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(54) Title: NON-HIV ANTIBODIES AS DISEASE MARKERS

(57) Abstract

The present invention relates to the detection of antibodies reactive with human endogenous retroviral antigens associated with various disease states. In particular, the invention provides immunoassay methods for the detection of such antibodies.

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NON-HIV ANTIBODIES AS DISEASE MARKERS

This disclosure is a continuation in part of patent application, U.S.S.N. 08/321,689, which is a continuation in part of U.S.S.N. 08/248,200, both of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention relates to the detection of antibodies reactive with human endogenous retroviral antigens associated with various disease states. The invention also relates to methods of treating disease by inhibiting the action of human endogenous retroviral genes and their products. In particular, the invention provides immunoassay methods for the detection of such antibodies.

Retroviruses are a group of RNA viruses characterized by the enzyme reverse transcriptase which generates complimentary DNA (cDNA) from the viral RNA. The cDNA is then copied and the double stranded DNA is incorporated into the host genome as a provirus. The proviral DNA, which is duplicated along with the host genome after each cell division, may remain latent for long periods of time before initiating transcription of the viral genes (Varmus, *Science*, 240:1427 (1988)).

In humans and other animals, proviral DNA can in some instances remain in the host germline DNA and be transmitted to subsequent generations as an endogenous retrovirus (ERV). After a number of generations the proviral DNA may undergo mutations so that it no longer encodes an infectious retrovirus. Such endogenous retroviruses have been implicated in a number of diseases such as cancer and autoimmunity (*see, e.g.*, Rasmussen *et al. Acta Neurol. Scand.* 88:190-198 (1993) and Venables *et al. Br. J. Rheum.* 31:841-846 (1992)).

A number of HERVs and HERV families have been identified. Examples include HERV 4.1 (Martin *et al., Proc. Natl. Acad. Sci. USA* 78:4892-4896 (1981)); RTLV-H Mager *et al., Proc. Natl. Acad. Sci. USA* 81:7510-7514 (1984)); HERV-K10 (HTDV-H) (Boller *et al. Virol.* 196:349-353 (1993)); HERV-K(C4) (Tassabehji *et al.*

Nuc. Acids. Res. 22:5211-5217 (1994)); HERV-P (HuERS and HuRRS-P) (Harada *et al.* 15:9153-9162 (1987)); and HERV-R (ERV-3) O'Connell *et al. Virol.* 138:225-235 (1984)).

Endogenous retroviruses are thought to be activated by a number of factors such as immune stimuli, steroid hormones, and chemical carcinogens. Some animals comprise ERVs capable of being expressed as infectious viral particles. Many HERVs are capable of protein expression and polypeptides encoded by HERVs have been isolated from human material.

Many HERVs display sequence homology to murine or primate retroviruses. A few have sequence homology to infectious human retroviruses such as human T cell leukemia virus types 1 and 2 (HTLV-1 and HTLV-II) (Perl *et al. Nuc. Acids. Res.* 17:6841-6854 (1989)).

Immunosuppressive polypeptides having sequences related to a retroviral transmembrane envelope protein from murine and feline leukemia viruses, p15E, have been detected in human tissues using monoclonal antibodies. p15E is a hydrophobic transmembrane protein (around 19,000 daltons) of the retroviral envelope which is synthesized as part of a precursor of molecular weight 80,000-90,000. Snyderman *et al.* (*Immunology Today* 5:240-244 (1984)) discuss p15E-like factors produced by tumor cells which depress macrophage-mediated functions. The expression of a p15E-like proteins may thus allow transformed cells to escape immune destruction. Snyderman *et al.* suggest that the immunosuppressive activity of p15E could be important in promoting tumorigenesis. p15E-like factors have been found in the serum and urine of patients suffering from malign and benign breast tumors (Stoger *et al.*, *Clin. Exp. Immunol.* 93:437 (1993)).

A central hydrophilic 26 amino acid region of p15E is thought to be important to the immunosuppressive properties of this protein (Schmidt *et al. Proc. Natl. Acad. Sci. USA* 84:7290 (1987)). This region of the protein has some sequence identity with sequences from HTLV, HIV, as well as human cytokines (Foulds *et al. Br. J. Cancer* 68:610-616 (1993) and Haraguchi *et al. J. Leukocyte Biol.* 52:469 (1992)).

Despite progress in the identification of HERVs and their connection to the pathogenesis of various diseases, no diagnostic assays based on the detection of HERVs gene products have been developed. Ideally, such assays should provide a low cost, safe

assay for the presence of particular markers which are highly associated with disease. The present invention addresses these and other needs.

SUMMARY OF THE INVENTION

The present invention provides methods of detecting antibodies reactive with an endogenous retroviral antigen associated with a disease state. In addition, the invention provides methods of treating disease associated with HERV expression by inhibiting HERV gene expression or the action of HERV gene products.

The assay methods comprise the steps of providing a biological sample suspected of comprising the antibody and contacting the sample with the endogenous retroviral antigen. The presence of the antibodies is determined by detecting the presence of an antigen-antibody complex.

A number of endogenous retroviral antigens associated with disease can be used in the assays of the invention. The retroviral antigens are typically immunosuppressive polypeptides such as p15E polypeptides. The p15E polypeptides may be isolated from appropriate tissue, chemically synthesized or recombinantly produced.

The assays are preferably performed on urine or oral fluid samples from subjects suspected of having a particular disease. The assays are usually immunoassays in which the HERV antigen is bound to a solid surface and the target antibodies are detected using standard techniques, such as with the use of labelled anti-human antibodies.

The method can be used for the early detection of a number of disease states, such as cancer, autoimmune diseases, and the like. Autoimmune diseases which can be detected include insulin-dependent diabetes mellitus, multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, or rheumatoid arthritis. Cancers include colon cancer, lung cancer, Hodgkin's disease, multiple myeloma, or breast cancer.

The methods of the invention may also include the detection of non-1/non-2 antibodies, that is antibodies which are cross-reactive with HIV-1 or HIV-2 envelope proteins in subjects who cannot be confirmed as being infected with HIV. The presence of these antibodies is indicative of the expression of an HERV antigen. To identify non-1/non-2 (*i.e.*, anti-HERV) antibodies, an enzyme immunoassay is used to detect antibodies reactive with HIV envelope proteins, such as gp160. Non-1/non-2 antibodies

are identified if the presence of HIV cannot be confirmed, *e.g.*, using a western blot assay.

Non-1/non-2 antibodies are preferably detected in urine.

The invention also provides kits for detecting the presence of an antibody associated with a disease state in a biological sample. The kits comprise an endogenous retroviral antigen capable of forming a complex with the antibody and a labelling system for detecting the presence of the antigen-antibody complex . The kits may further comprise a buffer solution for preparation of the sample. Typically, the retroviral antigen is a p15E polypeptide.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the results immunoassays of samples from subjects from low risk populations as compared to subjects diagnosed with various diseases. The number of subjects positive for non-1/non-2 antibodies or negative in all tests is presented.

Figure 2 shows the results of immunoassays of samples from subjects diagnosed as having various diseases. The number of subjects positive for non-1/non-2 antibodies or negative in all tests in each disease category is presented.

Figure 3 shows the results of immunoassays of samples from subjects diagnosed as having sexually transmitted diseases. The number of subjects positive for non-1/non-2 antibodies or negative in all tests is presented.

Figure 4 shows the results of competitive inhibition assays using an anti-p15E MAb in the urine immunoassays of the invention.

Figure 5 compares inhibition in the immunoassays using an ant-p15E MAb and an isotype matched non-relevant MAb.

Figure 6 shows the results of analysis of healthy subjects using urine immunoassays of the invention, except that the sample buffer was not used (back row in the bar graph). Also shown are results of the analysis of the same population using a p15E polypeptide as the antigen in the assay (front row in the bar graph).

Figure 7 shows results of assays detecting bovine and equine antibodies cross-reactive with gp160 in sera from these animals.

Figure 8 shows diurnal variation of urine antibodies against HERV antigens in three healthy subjects found to be negative in both urine western blot and standard serum immunoassays.

Figure 9 shows urine IgG levels in the subjects shown in Figure 8.

Figure 10 shows diurnal variation of urine antibodies against HERV antigens in an individual who was indeterminate in a serum western blot for HIV.

Figure 11 shows diurnal variation of urine antibodies against HERV antigens in an individual who was indeterminate in a serum western blot for HIV.

Figure 12 shows diurnal variation of urine antibodies against HERV antigens in an individual who was indeterminate in a serum western blot for HIV.

Figure 13 shows variation in oral fluid antibodies against HERV antigens in a patient with Parkinson's Disease. Samples were taken approximately every day for two months.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is based in part on the identification of subjects positive in immunoassays for the presence of antibodies reactive with HIV envelope proteins, but who cannot be confirmed as having an HIV infection by Western Blot. The "false positives" in the immunoassays are referred to here as having non-HIV-I/non-HIV-2 (non-1/non-2) antibodies. As used herein "non-1/non-2" antibodies or antisera are those specifically immunoreactive with a HIV envelope antigen (*e.g.* gp160) which are detected in subjects who cannot be confirmed to be HIV positive. Typically, HIV infection is confirmed by Western blot analysis as described below. Other methods of determining the presence of HIV infection (*e.g.*, viral isolation, PCR) can also be used. Since, as explained below, non-1/non-2 antibodies are immunologically reactive with HERV gene products, the presence of such antibodies can also be detected using HERV proteins or fragments thereof.

The presence of these anti-HERV or non-1/non-2 antibodies is shown here to be correlated with various diseases such as cancers, chronic renal failure, autoimmune diseases, and sexually transmitted diseases. The present invention provides methods for detection of such antibodies as useful markers for disease. In addition, inhibition of the expression or activity of the antigens recognized by non-1/non-2 antibodies can be used to treat disease.

Immunoassays

Typically, the immunoassays of the invention involve determining antibody levels in urine or other bodily fluids such as serum, oral fluid (*e.g.*, saliva), cerebrospinal fluid, semen, and the like. A variety of techniques for detecting antibodies can be used (*e.g.*, western blots, enzyme immunoassays). Any relevant glycoprotein or protein target can be used. For example, recombinantly produced retroviral envelope glycoproteins (*e.g.* HIV-1 gp160) which retain protein conformation and HERV cross-reactivity can be used. Alternatively, HERV gene products or fragments thereof can be used.

Samples from subjects confirmed to have non-1/non-2 antibodies can be used to identify HERV antigens useful in the assays of the invention. One of skill will recognize that a number of molecular biological techniques can be used to isolate HERV nucleic acids or polypeptides encoded by them. For a general discussion of standard molecular biological techniques *see*, Sambrook et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1982). For instance, expression libraries can be constructed using DNA isolated from subjects with non-1/non-2 antibodies. The expression libraries are then screened to identify clones expressing the polypeptides reactive with non-1/non-2 antibodies. Alternatively, sequences from previously identified HERVs can be used to identify the desired sequence using standard techniques, such as PCR and/or nucleic acid hybridization techniques.

Immunoassays for the detection of antibodies against particular HERV antigens, preferably p15E polypeptides or oligopeptides, are also provided. As used herein the terms "p15E polypeptide" and "p15E oligopeptide" refer to retroviral transmembrane envelope proteins from murine and feline leukemia viruses discussed above, as well as related oligopeptides isolated from humans and fragments thereof. p15E polypeptides can be identified by their ability to specifically bind monoclonal antibodies raised against p15E. Monoclonal antibodies specific for p15E are described in the literature and include 4F5, 19F8 (Cianciolo *et al.*, *J. Exp. Med.* 158:885 (1983) and Lostrom *et al.*, *Virology* 98:336 (1979)). A preferred monoclonal is 2B5 which is available from Biodesign International, Kennebunkport, Maine. Nucleic acids encoding p15E polypeptides are disclosed in Pallisgaard *et al. Nuc. Acids Res.* 17:6413 (1989).

The immunoassays of the invention are carried out using techniques well known in the art. (See for example, U.S. Patents 4,366,241; 4,376,110; 4,517,288; and

4,837,168). For a review of the general procedures of the invention, see also *Basic and Clinical Immunology* 7th Edition (D. Stites and A. Terr ed.) 1991.

The immunoassays can be either competitive or noncompetitive. In competitive binding assays, the sample analyte (in this case, target antibodies to a HERV) competes with a labeled analyte for specific binding sites on a capture agent (e.g., HERV antigens) bound to a solid surface. The concentration of labeled analyte bound to the capture agent is inversely proportional to the amount of free analyte present in the sample.

Noncompetitive assays are typically sandwich assays, in which the sample analyte (target antibody) is bound between two analyte-specific binding reagents. One of the binding agents is used as a capture agent and is bound to a solid surface. The other binding agent is labelled and is used to measure or detect the resultant complex by visual or instrument means.

A number of combinations of capture agent and labelled binding agent can be used. For instance, HERV antigens can be used as the capture agent and labelled anti-human antibodies specific for the constant region of human antibodies can be used as the labelled binding agent. Goat, sheep and other non-human antibodies specific for human immunoglobulin constant regions (eg. γ or μ) are well known in the art. Alternatively, the anti-human antibodies can be the capture agent and the antigen can be labelled.

Other proteins capable of specifically binding human immunoglobulin constant regions, such as protein A or protein G may also be used as the capture agent or labelled binding agent. These proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species. See, generally Kronval, et al., *J. Immunol.*, 111:1401-1406 (1973), and Akerstrom, et al., *J. Immunol.*, 135:2589-2542 (1985).

The non-competitive assays need not be sandwich assays. For instance, the antibodies in the sample can be bound directly to the solid surface. The presence of antibodies to the HERV in the sample can then be detected using labelled antigen.

Various immunoblot techniques (Western blot, slot-blot analysis) can also be used to confirm the presence of non-1/non-2 or anti-HERV antibodies in the sample. Western blots are the preferred method for confirming the presence of anti-HIV

antibodies in the sample after a sample is found to positive in an EIA. The technique generally comprises separating proteins by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support, (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with the separated proteins. This causes specific target antibodies present in the sample to bind their respective proteins. Target antibodies are then detected using labeled anti-human antibodies. This method of detecting target antibodies has the additional advantage of detecting antibodies to specific antigenic proteins.

Other assay formats include liposome immunoassays (LIA), which use liposomes designed to bind specific molecules (e.g., antibodies) and release encapsulated reagents or markers. The released chemicals are then detected according to standard techniques (see, Monroe et al., *Amer. Clin. Prod. Rev.* 5:34-41 (1986).

Some assay formats do not require the use of labelled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labelled and the presence of the target antibody is detected by simple visual inspection.

As mentioned above, depending upon the assay, various components, including the antigen, target antibody, or anti-human antibody, may be bound to a solid surface. Many methods for immobilizing biomolecules to a variety of solid surfaces are known in the art. For instance, the solid surface may be a membrane (*e.g.*, nitrocellulose), a microtiter dish (*e.g.*, PVC or polystyrene) or a bead (*e.g.*, magnetic beads). The desired component may be covalently bound or noncovalently attached through nonspecific bonding.

A wide variety of organic and inorganic polymers, both natural and synthetic may be employed as the material for the solid surface. Illustrative polymers include polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and the like. Other materials which may be employed, include paper, glasses, ceramics, metals, metalloids, semiconductive materials, cements or the like. In addition, are included substances that form gels, such as proteins (e.g., gelatins), lipopolysaccharides, silicates, agarose and polyacrylamides can be used.

Polymers which form several aqueous phases, such as dextrans, polyalkylene glycols or surfactants, such as phospholipids, long chain (12-24 carbon atoms) alkyl ammonium salts and the like are also suitable. Where the solid surface is porous, various pore sizes may be employed depending upon the nature of the system. In preparing the surface, a plurality of different materials may be employed, particularly as laminates, to obtain various properties. For example, protein coatings, such as gelatin can be used to avoid non-specific binding, simplify covalent conjugation, enhance signal detection or the like.

If covalent bonding between a compound and the surface is desired, the surface will usually be polyfunctional or be capable of being polyfunctionalized. Functional groups which may be present on the surface and used for linking can include carboxylic acids, aldehydes, amino groups, cyano groups, ethylenic groups, hydroxyl groups, mercapto groups and the like. The manner of linking a wide variety of compounds to various surfaces is well known and is amply illustrated in the literature. See for example Immobilized Enzymes, Ichiro Chibata, Halsted Press, New York, 1978, and Cuatrecasas, *J. Biol. Chem.* 245 3059 (1970).

In addition to covalent bonding, various methods for noncovalently binding an assay component can be used. Noncovalent binding is typically nonspecific absorption of a compound to the surface. Typically, the surface is blocked with a second compound to prevent nonspecific binding of labelled assay components. Alternatively, the surface is designed such that it nonspecifically binds one component but does not significantly bind another. For example, a surface bearing a lectin such as Concanavalin A will bind a carbohydrate containing compound but not a labelled protein that lacks glycosylation. Various solid surfaces for use in noncovalent attachment of assay components are reviewed in U.S. Patent Nos. 4,447,576 and 4,254,082.

Many assay formats employ labelled assay components. The labelling systems of the invention can be in a variety of forms. The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. A wide variety of labels may be used. The component may be labelled by any one of several methods. The most common method of detection is the use of autoradiography with ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P labelled compounds or the like. Non-radioactive labels include ligands which bind to labelled antibodies, fluorophores, chemiluminescent agents, enzymes, and antibodies which can serve as specific binding

pair members for a labelled ligand. The choice of label depends on sensitivity required, ease of conjugation with the compound, stability requirements, and available instrumentation.

Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (e.g., biotin) is covalently bound to the molecule. The ligand then binds to an anti-ligand (e.g., streptavidin) molecule which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-ligands can be used. Where a ligand has a natural anti-ligand, for example, biotin, thyroxine, and cortisol, it can be used in conjunction with the labelled, naturally occurring anti-ligands. Alternatively, any haptenic or antigenic compound can be used in combination with an antibody.

The molecules can also be conjugated directly to signal generating compounds, e.g., by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescent compounds include luciferin, and 2,3-dihydrophthalazinediones, e.g., luminol. For a review of various labelling or signal producing systems which may be used, see, U.S. Patent No. 4,391,904.

Nucleic acid hybridization assays

In addition to immunoassays, HERV nucleic acid sequences can be detected using standard hybridization techniques. For example oligonucleotide probes specific for particular HERV nucleic sequences (*e.g.*, DNA or mRNA) can be designed to detect the presence of these sequences.

A variety of methods of specific DNA and RNA detection using nucleic acid hybridization techniques are known to those of skill in the art. For example, the presence or absence of a target DNA in a sample typically involve blotting techniques, such as Southern blotting. Alternatively, a Northern transfer may be used for the detection of mRNA in samples of RNA. RNA may also be detected using labeled complementary probes in solution, such as RNase protection methods. Hybridization

techniques are generally described in "*Nucleic Acid Hybridization, A Practical Approach*," Ed. Hames, B.D. and Higgins, S.J., IRL Press, 1985; Gall and Pardue (1969), *Proc. Natl. Acad. Sci.*, U.S.A., 63:378-383; and John, Burnsteil and Jones (1969) *Nature*, 223:582-587.

Typically labelled signal nucleic acids are used to detect hybridization. Complementary nucleic acids or signal nucleic acids may be labelled by any one of several methods typically used to detect the presence of hybridized polynucleotides. The most common method of detection is the use of autoradiography with ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P-labelled probes or the like. Other labels include ligands which bind to labelled antibodies, fluorophores, chemiluminescent agents, enzymes, and antibodies which can serve as specific binding pair members for a labelled ligand.

Detection of a hybridization complex may require the binding of a signal generating complex to a duplex of target and probe polynucleotides or nucleic acids. The label may also allow indirect detection of the hybridization complex. For example, where the label is a hapten or antigen, the sample can be detected by using antibodies. In these systems, a signal is generated by attaching fluorescent or enzyme molecules to the antibodies or in some cases, by attachment to a radioactive label. (Tijssen, P., "Practice and Theory of Enzyme Immunoassays," *Laboratory Techniques in Biochemistry and Molecular Biology*, Burdon, R.H., van Knippenberg, P.H., Eds., Elsevier (1985), pp. 9-20.)

The sensitivity of the hybridization assays may be enhanced through use of a nucleic acid amplification system which multiplies the target nucleic acid being detected. Examples of such systems include the polymerase chain reaction (PCR) system and the ligase chain reaction (LCR) system. Other methods recently described in the art are the nucleic acid sequence based amplification (NASBA™, Cangene, Mississauga, Ontario) and Q Beta Replicase systems. For a general overview of PCR see *PCR Protocols: A Guide to Methods and Applications*. (Innis, M, Gelfand, D., Sninsky, J. and White, T., eds.), *Academic Press*, San Diego (1990).

HERV expression and disease

Without wishing to be bound by theory, it is believed that HERV expression is an important element in pathogenesis of a number of diseases. In particular, HERVs having immunosuppressive abilities, such as those related to p15E,

allow certain cells (*e.g.*, virally infected, neoplastic cells, self-reactive lymphocytes) to escape normal immune surveillance by cytotoxic T cells and other elements of the immune system. In the absence of a mechanism for controlling their growth, the cells proliferate and the disease progresses. Polypeptide products of HERV gene expression, however, may have pleiotropic effects. Thus, in addition to immune suppression, the polypeptides may be more directly involved in the etiology of disease. For instance, neurotoxic HERV gene products may be involved in degenerative nervous system diseases. Alternatively, HERV gene expression may be a marker for rearrangement events which cause disease.

In the cases where HERV expression is an early event in the development of the disease, detection of the HERVs or anti-HERV antibodies using the assays of the invention provides a useful method for early detection of a number of diseases. In addition, anti-HERV antibodies can be used to monitor the success of therapeutic protocols.

Since HERV expression is associated with pathogenesis, methods for inhibiting HERV gene expression or inhibiting the action of HERV gene products can be used to treat disease. For instance, compounds which inhibit retroviral transcription can be used to treat diseases associated with HERV gene expression. In addition, methods of inhibiting translation of HERV mRNA transcripts, such as antisense techniques can also be used. Finally, where the gene products bind particular receptors, analogs of the HERV polypeptides or their receptors can be used to block the interaction and thus prevent the cellular response to HERV polypeptides. Thus, for instance, antagonists of P15E, which block binding of the protein to cellular receptors can be used. In addition, since some retroviral products are superantigens, agents that inhibit the action of superantigens (*e.g.*, inhibit the interaction of the molecule with either T cell receptors or MHC molecules) can be used.

The detection of non-1/non-2 or anti-HERV antibodies in a subject can be associated with a number of diseases. For example, risk for autoimmune diseases such as systemic lupus erythematosus, immune dependent diabetes mellitus type II, scleroderma, Sjogren's syndrome, CFIDS, autoimmune hemolytic anemia, alopecia, idiopathic thrombocytopenic purpura, autoimmune thyroiditis, glomerulonephritis, uveitis, and the like can be detected using the assays of the invention or treated using antiviral therapies. In addition, neurological diseases such as myasthenia gravis or

rheumatoid arthritis can also be treated. Similarly, anti-viral compounds can be used to treat a number of cancers such as colorectal, gastric, lung, breast, multiple myeloma, Hodgkin's disease, and head and neck cancers. The assays of the invention can be used to identify individuals at risk for these diseases, as well.

Expression of HERV antigens may also be implicated in the progression from latent HIV infection to acquired immune deficiency syndrome (AIDS). A growing body of evidence suggests that HIV does not act alone to cause AIDS and that certain co-factors may be required for pathogenesis. Immunosuppressive HERV antigens can be involved in this process. Thus, detection of anti-HERV antibodies can be an early sign of AIDS progression. In addition, specific inhibition of HERVs can be used to treat HIV infection and other immune deficiency diseases.

Moreover, detection of antibodies in different bodily fluids can reveal information about compartmentalization of the immune response to the HERV antigens. For instance, detection of antibodies in urine in serum negative individuals indicates that HERV expression is localized in the urogenital tract. Alternatively, antibodies detected in oral fluid may indicate expression in the head or neck. This in turn reveals information about sites of pathogenesis. For instance, data presented below indicates that detection of anti-HERV antibodies in oral fluids is associated with Parkinson's disease.

The invention provides methods of treating HIV infection, autoimmune diseases, cancers and other disease associated with HERV gene expression. Generally, the therapeutic methods rely on antiviral therapies designed to interfere with replication of retroviruses or inhibit activity of the gene products. For instance, compounds known to inhibit retroviral replication by interfering with reverse transcriptase activity can be used. Such compounds include AZT, another nucleoside analogs such as dideoxyinosine (ddI), and dideoxycytosine (ddC). Other targets include the regulation of the synthesis, maturation and transport from the nucleus to the cytoplasm of viral RNA from proviral DNA. For example compounds that inhibit the activity of regulatory proteins such as *tat* or *rev* can be used. Translation of viral RNA can be inhibited using antisense technology. In addition, if posttranslational processing of polyproteins is required to produce the HERV antigen of interest, inhibition of retroviral proteases can also be used. For a discussion of various antiviral compounds that can be used to inhibit HERV expression, *see*, White and Fenner *Medical Virology*, 4th ed., esp. Chap. 16 (Academic Press, San Diego, CA 1994). Alternatively, the compounds can inhibit HERV gene

product activity by interfering with the interaction of the protein with various cellular proteins. For instance, as discussed above, analogs of P15E which antagonize the interaction of that protein with cellular proteins can be used.

Antiviral compounds used to treat the diseases noted above can be prepared in pharmaceutical compositions and administered using methods well known in the art. Depending upon the particular drug being administered, the pharmaceutical compositions are generally intended for parenteral, topical, oral or local administration for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include powder, tablets, pills, and capsules.

Evidence provided here indicates that HERV expression can undergo diurnal variation. Thus, the timing of antiviral therapies is an important aspect of the administration of such compounds. Preferably, the levels of antibodies against particular endogenous retroviral antigens is monitored in a patient throughout a 24 hour period to determine what time periods show increased antibody titres. Timing of the administration of the antiviral compound or other compound is then appropriately selected to be most effective. Typically, the compound is administered to be concurrent with or slightly prior to the period of increased antibody levels. Generally, samples showing increased antibody levels are those having antibody levels above a predetermined cut-off (*e.g.*, at least about twice that of the median level detected throughout the day). Antibody levels are typically measured using the immunoassays described below. Suitable pharmaceutical formulations for use in the present invention are found in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985). A variety of pharmaceutical compositions comprising compounds and pharmaceutically acceptable carriers can be prepared.

Injectable preparations, for example, sterile injectable aqueous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may

be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectable.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings. Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

The antiviral compounds can also be administered in liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., *Ann. Rev. Biophys. Bioeng.* 9:467 (1980), U.S. Pat. Nos. 4, 235,871, 4,501,728 and 4,837,028.

The pharmaceutical compositions containing the compounds can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a patient already suffering from a disease, as described above, in an amount sufficient to decrease anti-HERV titres and preferably cure or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on the compound being administered, the severity of the disease, the weight and general state of the patient and the judgement of the prescribing physician.

The following examples are provided to illustrate and not limit the scope of the invention disclosed here.

Example 1

Test for Non-1/Non-2 Antibodies

This example provides the results of urine immunoassays in which the correlation between various diseases and non-1/non-2 antibodies is demonstrated.

The presence of non-1/non-2 antibodies was determined by the detection of antibodies to HIV in urine, using enzyme immunoassay (EIA) procedures. For confirmation, specimens repeatedly reactive in the EIA were further tested by a Western blot procedure. All urine specimens were handled by established, good laboratory working practices and the CDC guidelines for working with HIV material. (Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services, 1988, Publication No. (NIH) 88-8395).

1. Enzyme Immunoassay

The EIA employed an HIV-1 recombinant envelope glycoprotein, gp160, expressed in insect cells. The fall army worm cell line, SF9, was transfected with a recombinant baculovirus vector comprising the full length gp160 gene according to standard techniques (*see, e.g.*, Smith *et al.*, *Proc. Natl. Acad. Sci. USA* 82:8404-8408 (1985) and WO 92/22654).

The recombinant envelope protein was then adsorbed onto the wells of microwell strip plates and used in assays generally as follows. Sample buffer and urine specimens or urine controls were added to the wells and incubated. Antibodies in the samples were visualized using a conjugate, consisting of alkaline phosphatase chemically bound to goat antihuman immunoglobulin antibodies. P-nitrophenylphosphate (PNPP), the substrate for the enzyme, was added to all wells and incubated. If antibodies to HIV-1 were present in the sample, the enzyme produced a color change from colorless to yellow. The intensity of the color was determined to be proportional to the amount of HIV-1 antibodies present in the test sample. The reaction was terminated by the addition of a stop solution containing ethylenediaminetetraacetic acid (EDTA). The absorbance values are determined spectrophotometrically with a plate reader at a wavelength of 405 nm.

The following reagents were used.

Sample Buffer - goat IgG, bovine IgG, horse IgG, 0.01% v/v, 9% serum (3% horse, 3% bovine, 3% goat) in 0.05M Tris-HCl buffer pH 7.2 and 0.15 M sodium chloride. If desired, the immunoglobulins can be coated on polystyrene beads.

Positive Control - Human urine containing antibody to HIV-1 and 0.1% sodium azide as preservative.

Negative Control - Human urine negative for HIV-1 and HIV-2 antibodies containing 0.1 % sodium azide as preservative.

Conjugate Solution - Alkaline phosphatase labeled goat antihuman immunoglobulin in Tris buffered saline containing bovine serum albumin and less than 0.1% sodium azide as preservative.

Substrate Solution - P-nitrophenylphosphate (PNPP) in diethanolamine buffer, containing magnesium chloride and 0.1% sodium azide as preservative.

Stop Solution - Solution containing ethylenediaminetetraacetic acid (EDTA).

Wash Solution (10X) - Tris-buffered saline containing NP-40 and 1.0% sodium azide as preservative.

Specimens were collected in accordance with approved standards. Specimens excessively contaminated with bacteria, blood, or sediment were not used because of inconsistent test results. The urine samples were refrigerated at 2-8°C and were tested as soon after collection as possible. Specimens to which a urine preservative has been added may be stored at room temperature (15-30°C). For instance, urine to which Stabilur urine preservative (R.P. Cargille Laboratories, Inc., Cedar Grove, NJ) has been added can be used successfully in the assay.

PROCEDURE

25 µl of Sample Buffer was added to each well. Next, 200 µl of each specimen or control was added to the appropriate wells. 2 positive and 3 negative controls were assayed with each plate or partial plate of test samples. The plates were incubated at 37°C ± 1°C for 60 minutes.

At the end of the incubation, the solution was completely aspirated from the wells by using a plate washer or a hand held aspirator connected to a vacuum source. The wells were then filled with 1X Wash Solution and immediately aspirated. This step was repeated 5 times.

The plates were blotted by inverting on clean absorbent towels and 100 μl of Conjugate Solution was added to each well containing a specimen or control. Each plate was sealed and incubated at 37°C ± 1°C for 60 minutes.

At the end of the incubation, the Conjugate Solution was completely aspirated from the wells and the wells were washed with the 1X Wash Solution. Next, 100 μl of Substrate Solution was added to each well containing a specimen or control. Each plate was covered and incubated at 37°C ± 1°C for 30 minutes.

At the end of the incubation, 50 μl of Stop Solution was added to each well containing a specimen or control. A microplate reader was used to read the plates at 405 nm. Plates were read within 30 minutes of adding the Stop Solution.

The absorbance value of each Negative Control well was preferably not greater than 0.200. One Negative Control value was discarded if outside this range and the mean calculated by adding the two remaining absorbance values and dividing by 2. If two or more Negative Control values were greater than 0.200, the assay was determined to be invalid and was repeated.

Each Positive Control had an absorbance value equal to or greater than 0.900. No Positive Control value was discarded. If any Positive Control value was less than 0.900 the assay was determined to be invalid and was repeated.

The cutoff value for determining a sample was positive was 0.180 plus the mean absorbance of the Negative Controls. Samples with absorbance values greater than or equal to the cutoff value were considered initially reactive. All initially reactive samples were retested in duplicate using another aliquot of the original specimen. If after repeat testing, the absorbance values of both duplicate samples were less than the cutoff value, then the original specimen was considered to be a non-repeatable reactive sample and negative for antibodies.

If after repeat testing, the absorbance value of one or both of the duplicates was equal to or greater than the cutoff value, the sample was considered repeatably reactive and potentially positive for antibodies to HIV-1.

2. Western Blot

Bio-Rad Novapath HIV-1 Immunoblot Test (Bio-Rad, Inc. Richmond, California) was used generally according to manufacturer's instructions. In the assays, a test batch was defined as a set of no more than 10 or 30 immunoblot strips depending on

the kit size utilized. A non-reactive serum kit control (NR) and 2 levels of reactive serum kit controls (R1 and R2) were included with each serum test batch.

Procedures for testing human serum or plasma were according to manufacturer's instructions. The methods were modified as follows, the working wash/diluent was prepared as needed at 1X the day of use. In addition, the steps which succeed aspiration of the final water rinse from the troughs were as follows. The trays were covered with paper towels and strips were allowed to air-dry before recording results (strips generally require overnight air-drying). When strips were dry, results were recorded on a Serum Western Slot Test Results chart according to the instructions. For urine samples, a Negative (non-reactive) Urine Control, 2 levels of reactive urine controls, a non-reactive serum kit control (NR) and a weak reactive serum kit control (R2) were included with each urine test batch.

The manufacturer's instructions were followed with the following modifications. 2 ml Working Wash/Diluent was added to troughs to which serum kit controls were added. 1 ml Working Wash/Diluent was added to troughs to which the Negative Urine Control, Positive Urine Control or the urine test samples were added. 1.8 ml Working Wash/Diluent was added to the trough to which the Low Positive Urine Control was added.

The trays were covered and placed on a rocker for 5 minutes before adding subject or control samples. Trays were rocked at an angle of approximately 7 degrees at 15-18 cycles per minute. After rocking, 20 μl of NR (nonreactive) serum kit control was added to the small segment formed by the two ridges at the numbered end of a trough containing 2 ml of Working Wash/Diluent for a 1/100 dilution.

Using the same procedure, the following steps were solutions were added: 20 μl of R2 (weak reactive) serum kit control was added to a separate trough containing 2 ml of Working Wash/Diluent for a 1/100 dilution. 1 ml of Negative Urine Control was added to a trough containing 1 ml of Working Wash/Diluent for a 1/2 dilution. 1 ml of Positive Urine Control was added to a separate trough containing 1 ml of Working Wash/Diluent for a 1/2 dilution. 0.2 ml of the Low Positive Urine Control was added to a trough containing 1.8 ml of Working Wash/Diluent for a 1/50 dilution. 1 ml of urine test samples was added to troughs containing 1 ml of Working Wash/Diluent for a 1/2 dilution of each urine test sample.

The trays were covered and incubated on rockers overnight (18-22 hours) at room temperature (15-30C°). After incubation, the troughs were aspirated and 5 ml Working Wash/Diluent was added to each trough. The trays were recovered and incubated on rockers for 10 minutes at room temperature. The aspiration and incubation steps were repeated.

Troughs were then aspirated completely and 2 ml of Anti-Human IgG Enzyme Conjugate was added to each trough and incubated on rockers for 30 minutes at room temperature. Troughs were aspirated again and 5 ml Working Wash/Diluent was added to each trough. Trays were incubated on rockers for another 10 minutes at room temperature. The aspiration and incubation steps were repeated.

Troughs were aspirated completely and 2 ml Substrate Solution was added to each trough and incubated on rockers for 10 minutes at room temperature. Troughs were aspirated, approximately 5 ml high purity water was added to each trough, and the trays were incubated on rockers for 5 minutes at room temperature. The aspiration and incubation steps were repeated.

The troughs were aspirated and the trays were covered with paper towels and strips were allowed to air-dry before recording results (strips generally require overnight air-drying).

For quality control, the Negative (non-reactive) Urine Control must not exhibit any bands or background. The Low Positive Urine Control must exhibit the following band profile: The gp120 band must be distinct but significantly less reactive than the gp160 band. ENVELOPE bands must be typical in appearance, i.e. diffuse. The immunoblot must also exhibit at least 1 band representing gene products from each of the POLYMERASE (p65, p51, p32) and GAG (p55, p24, p18) regions. These bands must exhibit reactivity equivalent to or exceeding the reactivity of the gp120 band.

The Positive Urine Control typically exhibited reactivity exceeding that of the Low Positive Urine Control at the gp160, gp120, p65, p55, p51, gp41-43, p32, p24 and p18 band positions. It also exhibited various non-specific bands. Serum kit controls NR and R2 were included with urine test batches as an additional check of test kit functionality. The NR serum kit control typically exhibited various immunoreactive bands and/or non-specific bands. Generally, the reactivity of these bands was weaker than that associated with the gp120 band of immunoblots incubated with Low Positive Urine Controls. ENVELOPE region bands (gp160, gp120, gp41-43), if present, were

atypical in appearance (thin lines). The NR serum kit control typically exhibited no bands on immunoblots. The R2 serum kit control typically exhibited reactivity at the gp160, gp120, p65, p55, p51, gp41-43, p32, p24, and p18 band positions. These bands generally exceeded the reactivity associated with the gp120 band of immunoblots incubated with Low Positive Urine Controls. The R2 serum kit control also exhibited some degree of reactivity at the p15 band position as well as various non-specific bands.

The results of urine samples were interpreted and recorded as follows. A positive test result is indicated by reactivity to gp160 only and/or reactivity to any two of the following products: p24; gp41-43; gp120/gp160 or gp160 only. A negative test result is indicated by the absence of any bands on an immunoblot strip. An indeterminate test result is indicated by any pattern of one or more bands that does not meet the positive criteria. Samples that were repeatedly reactive in the EIA but negative for envelope glycoproteins in the Western blot were considered to contain non-1/non-2 antibodies. Further conformation by isotype analysis can also be preformed.

The results of these experiments are shown in Figures 1-3. Finally, Figure 1 shows that only 0.6 percent of over 3800 low risk subjects were positive for non-1/non-2 antibodies. In contrast, 27 percent of subjects diagnosed with various diseases were positive for non-1/non-2 antibodies. These results demonstrate that detection of non-1/non-2 antibodies are an early indication that a HERV antigen is being expressed and that the subject is at risk for disease.

Figure 2 shows the number of subjects diagnosed as having particular diseases, which were positive for non-1/non-2 antibodies or were negative in both the EIA and Western blot. As can be seen, a significant proportion of the subjects in each disease category were positive for non-1/non-2 antibodies.

Figure 3 shows the same results for subjects diagnosed with sexually transmitted diseases (STDs). 21 percent of the 156 subjects tested were positive for non-1/non-2 antibodies. These results suggest that STD microorganisms may be associated with activation of HERV expression.

Example 2

Competitive inhibition of EIA with a p15E monoclonal antibody

This example shows that a monoclonal antibody specific for p15E, inhibits binding of urine non-1/non-2 antibodies to HIV gp 160 in subjects that have been shown

to have non-1/non-2 antibodies. Detection of antibodies reactive with p15E polypeptides are thus useful in the early diagnosis of disease.

In these experiments, the ability of 2B5 (available from Biodesign International, Kennebunkport, ME) to inhibit binding of urine antibodies was determined in a competitive inhibition EIA. The EIA employed was the same as described above except for the following modifications. The sample buffer was 10% Bovine Serum Albumin (BSA) in Tris buffered saline (BSA-Tris). In the first step of the test procedure, 5μl of MAb 2B5 (200μg/ml) and 200 μl of test urine were mixed. Next, 20 μl of BSA-Tris was added to each well followed by 200 μl of the 2B5 and test urine mixture. A control non-relevant, monoclonal antibody, 451, was also used at the same concentrations. This monoclonal is a mouse anti-HIV gp120 antibody, which is of the same isotype as the MAb 2b5 (IgG_1) and was previously shown not to react with the antigen used in the assays described above. The incubations and other assay procedures were as described above.

Figure 4 shows the results of these experiments. In 2 of 24 samples tested, the 2B5 antibody significantly inhibited binding. Figure 5 shows that in the two samples that showed inhibition by 2B5, inhibition was greater than that for the control MAb.

Example 3

Sample buffer inhibits detection of anti-HERV antibodies

This example provides evidence that in the absence of the sample buffer described in Example 1, above, high levels of non-1/non-2 antibodies are detectable in the urine of healthy subjects.

Figure 1, above, shows that about 0.6% of healthy subjects were positive for non-1/non-2 antibodies when samples were assayed using the sample buffer described there. Figure 6 shows the results of analysis of healthy subjects using urine immunoassays as described above, except that the sample buffer was not used (back row in the bar graph). Using an OD of 0.45 as a cut off for positive results, it can be seen that approximately 11% (9/82) tested positive for urine retroviral antibodies (*i.e.*, non-1/non-2 antibodies).

Figure 6 also shows the results of the analysis of the same population using a p15E oligopeptide as the antigen in the assay (front row in the bar graph). As in the assays described above the sample buffer comprising animal sera was not used. The

peptide antigen was the synthetic sequence: LQNRRGLDLLFLKEGGL, CKS-17 (*see*, Cianciolo *et al. Science* 230:453-455 (1985)). As can be seen in Figure 6, reactivity with p15E was also detected in healthy subjects.

Thus, these results show that in the absence of sample buffers designed to reduce background in the assays, a larger proportion of healthy subjects (11% as compared to 0.6%) show reactivity with HIV antigens.

As noted above, the sample buffer used in the assays of Example 1 contains horse, bovine, and goat sera. The data presented in Figure 7 are the results of experiments using anti-bovine and anti-equine antibodies to detect bovine and equine antibodies bound to the HIV antigens used in the assays described above. Briefly, gp160 was coated on microwells and bovine or horse sera or controls were incubated in the microwells for 60 minutes at 37°C and then washed with Tris-buffered saline, 0.1% NP-40. Alkaline phosphatase labeled goat anti-bovine or anti-horse IgG was then added and incubated for 60 minutes at 37°C. Alkaline phosphatase activity was the detected according to standard techniques.

These results show that antibodies in the animal sera (presumably reactive with animal endogenous retroviruses) are cross-reactive with the epitopes on the HIV antigens used in the assay. These epitopes are the same as those recognized by the human anti-HERV antibodies (*i.e.*, non-1/non-2 antibodies) present in the sample. Thus, samples from human subjects which are anti-HERV antibody positive in immunoassays using the sample buffer comprise high antibody titres and/or high affinity antibodies that out-compete the cross-reactive antibodies in the animal sera.

In addition, the assays using a p15E polypeptide as an antigen show that reactivity to this peptide can be found in healthy subjects. These data support the results shown in Example 2, showing that anti-p15E antibodies inhibit binding of non-1/non-2 antibodies.

Example 4

Diurnal Variation in non-1/non-2 antibodies

This example provides evidence that using the assays described in Example 3, above, diurnal variation in non-1/non-2 antibody levels sometimes can be detected. These results indicate diurnal variation in expression of HERV antigens, as well.

Figure 8 shows diurnal variation in three healthy subjects found to be negative in both urine western blot and standard serum immunoassays. Urine samples were collected seven times over the course of a day. Each sample was assayed using the methods described in Example 3, above. As can be seen from Figure 8, although the antibodies levels were low in all three subjects, variability was detected.

Figure 9 shows urine IgG levels in the same three subjects. IgG levels were determined using radial immunodiffusion kits as supplied by the Binding Site Inc., San Diego, CA. One of the subjects, #617, showed a peak of IgG concentration early in the day that did not appear to be associated with increases in retroviral urine antibodies shown in Figure 8.

Figure 10 shows similar results of a fourth individual, #616, who was indeterminate in a serum western blot for HIV. The western blot bands which showed reactivity as identified in the figure. The IgG concentration at each of the collection times was determined and is shown along the X axis. As can be seen in this figure, the third urine collection of the day showed increased IgG concentration and increased level of retroviral urine antibodies. No reactivity against p15E was found.

Another subject, #647, who was also serum western blot indeterminate, showed diurnal variation against both retroviral antigens and p15E throughout the day (Figure 11). Urine IgG concentrations are also shown. Some correlation IgG concentrations and urine retroviral antibodies is seen. For example the 1:30 collection time showed increased IgG and retroviral antibody levels.

The subject whose results are shown in Figure 12 had lower overall urine IgG levels than #647. This subject, however, had distinct peaks of retroviral urine antibody reactivity about midday.

Figure 13 shows the results of immunoassays of oral fluid samples taken from a patient with Parkinson's disease over the course of approximately two months. As can be seen there, HERV and P15E antibody levels varied dramatically over the time period.

Example 5

Urine Antibodies to HERVs in Healthy and HIV-1 Seropositive Subjects

This example provides evidence of the differential occurrence of urine antibodies in four patient groups: Healthy (H): healthy low risk subjects with no serum or urine antibodies (WB) to HIV-1; Healthy-Sero-Indeterminate (HSI): healthy low risk subjects who were serum WB indeterminate; HIV-Asymptomatic (HIV-A): HIV- 1 seropositive positive subjects. HIV-Symptomatic (HIV-S): subjects serum positive for HIV-1 antibody with AIDS defining illnesses. Blood samples taken when subjects were admitted to the study were tested for antibody to HIV-1 by the HIV-1 EIA test (Abbott) and confirmed by Western Blot (Biorad).

Urine specimens were voided serially into separate cups over 24 hours and included a morning void for the following day. Total IgG concentration was determined for each void. Three antigens were used for urine antibody assays. recRVgp is a recombinant high molecular weight envelope glycoprotein (Calypte Biomedical) that shares epitopes with several HERVs; CKS-17; and pepHERV 4.1, a synthetic peptide deduced from the *env* region of HERV 4.1 (Martin *et al. Proc. Natl. Acad. Sci. USA* 78:4892-4896 (1981)). Its sequence is as follows: QNRLALDYLLAAEGGVC. Urine specimens were tested for antibody by the EIA techniques described in Example 1.

None of the urine specimens (Table 1) from the H group had antibodies to the test antigens (total of 49 voids obtained from 7 subjects). In the HSI group each of the 8 subjects had antibody to pepHERV 4.1 in at least one void, 4/8 had antibody to CKS-17, and 7/8 were positive to recRVgp. Three of the 18 HIV-A patients had been HIV-1 serum positive for not less than 7 years. In the HIV-A group 132/144 voids had antibodies in the urine to recRVgp. All were negative for antibodies to CKS-17 and pepHERV 4.1. In the HIV-S category 8/8 were urine positive for antibody to recRVgp (77/81 total voids). All 8 in this group had at least one void positive for antibody to pepHERV 4.1 (33/81 voids). Three had 1 void containing antibody to CKS-17 (5/81 voids). There was no correlation with HERV urine antibody reactivity and total IgG concentration in the urine.

For each category of HIV-1 infected patients in which HERV antibodies were found, the frequency of reactivity to pepHERV 4.1 was twice that of CKS-17. Importantly, urine antibodies to HERVs were not detected in healthy low risk subjects presumed not to be infected by HIV-1. The detection of urine antibodies to pepHERV 4.1 and CKS-17 in the healthy serum-indeterminate group may correlate with the

indeterminate WB serum tests found in this population category. This finding may result from a cross reactivity between endogenous and exogenous retroviruses.

The absence of urine antibodies to pepHERV 4.1 and CKS-17 in the asymptomatic HIV-1 exposed group has a number of possible explanations. As one example, the inflammatory response that appears to activate HERVs and the ensuing HERV antibody response during AIDS defining symptomatic periods, may not have been sufficient in the asymptomatic HIV-1 group. These results show that the urine antibody response to HERVs can serve as a surrogate marker for HIV-1 disease progression or be applicable in the evaluation of HIV-1 therapeutic modalities. Thus, these tests provide a simple and sensitive method to detect a number of diseases.

Table 1: HERV Urine Antibodies in Healthy and HIV-1 Seropositive Subjects

Subject Category[1]	Number of Subjects	Number of Voids Tested	Urine Antibody Response[2] to: Recombinant: recRVgp	Synthetic peptide: pepHERV 4.1	Synthetic peptide: CKS-17
H	7	49	0	0	0
HSI	8	60	7	8	4
HIV-A	18	144	18	0	0
HIV-S	8	81	8	8	3

1 H, healthy, low risk subjects with no evidence of HIV-1 infection; HSI, healthy low risk sero-indeterminate: HIV-A, healthy, asymptomatic, HIV-1 serum antibody positive; HIV-S, AIDS defining symptomatic and HIV-1 serum antibody positive.

2 Number of subjects with at least 1 urine void positive to any of the target antigens.

The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference.

WHAT IS CLAIMED IS:

1. A method of detecting an antibody reactive with an endogenous retroviral antigen associated with a disease state, the method comprising the steps of:

providing a biological sample suspected of comprising the antibody;

contacting the sample with the endogenous retroviral antigen; and

detecting the presence of an antigen-antibody complex.

2. The method of claim 1, wherein the antigen is a p15E oligopeptide.

3. The method of claim 2, wherein the p15E oligopeptide is CKS-17.

4. The method of claim 1, wherein the antigen is a pepHERV 4.1 oligopeptide.

5. The method of claim 1, wherein the antigen comprises an epitope specifically immunoreactive with monoclonal antibody 2B5.

6. The method of claim 1, wherein the biological sample is a urine sample.

7. The method of claim 1, wherein the biological sample is an oral fluid sample.

8. The method of claim 1, wherein the step of detecting is carried out using an anti-human antibody linked to a label.

9. The method of claim 8, wherein the label is a detectable enzyme.

10. The method of claim 1, wherein the disease state is an autoimmune disease.

11. The method of claim 10, wherein the autoimmune disease is insulin-dependent diabetes mellitus type I, systemic lupus erythematosus, or rheumatoid arthritis.

12. The method of claim 1, is a neurological disease.

13. The method of claim 12, wherein the disease is multiple sclerosis, myasthenia gravis, or Parkinson's disease.

14. The method of claim 1, wherein the disease state is cancer.

15. The method of claim 14, wherein the cancer is colon cancer, lung cancer, or breast cancer.

16. The method of claim 1, wherein the antigen is bound to a solid surface.

17. The method of claim 1, further comprising detecting non-1/non-2 antibodies in the sample.

18. The method of claim 17, wherein the non-1/non-2 antibodies are detected using an enzyme immunoassay and confirmed using a western blot assay.

19. The method of claim 17, wherein the non-1/non-2 antibodies are detected in urine.

20. The method of claim 17, wherein the non-1/non-2 antibodies are detected using an HIV envelope glycoprotein.

21. The method of claim 20, wherein the envelope glycoprotein is gp160.

22. The method of claim 21, wherein the gp160 is recombinantly produced.

23. A kit for detecting the presence of an antibody associated with a disease state in a biological sample, the kit comprising an endogenous retroviral antigen capable of forming a complex with the antibody, and a labelling system for detecting the presence of the antigen-antibody complex.

24. The kit of claim 23, further comprising a buffer solution for preparation of the sample.

25. The kit of claim 23, wherein the endogenous retroviral antigen is a p15E oligopeptide.

26. The kit of claim 23, wherein the endogenous retroviral antigen is a pepHERV 4.1 oligopeptide

27. The kit of claim 23, wherein the labelling system includes a detectable enzyme.

28. A method of treating a disease by inhibiting human endogenous retrovirus expression in a patient, the method comprising administering to the patient a therapeutically effective dose of an antiviral compound.

29. The method of claim 28, wherein the disease is an autoimmune disease.

30. The method of claim 28, wherein the disease is a neurological disease.

31. The method of claim 28, wherein the disease is an immune deficiency disease.

32. The method of claim 28, wherein the disease is cancer.

33. The method of claim 28, wherein the antiviral compound is administered when endogenous retroviral expression is increased.

34. A method of treating a disease by inhibiting activity of human endogenous retroviral gene product in a patient, the method comprising administering to the patient a therapeutically effective dose of a compound capable of inhibiting activity of human endogenous retroviral gene product .

35. The method of claim 34, wherein the disease is an autoimmune disease.

36. The method of claim 34, wherein the disease is a neurological disease.

37. The method of claim 34, wherein the disease is an immune deficiency disease.

38. The method of claim 34, wherein the disease is cancer.

39. The method of claim 34, wherein the compound is administered when endogenous retroviral expression is increased.

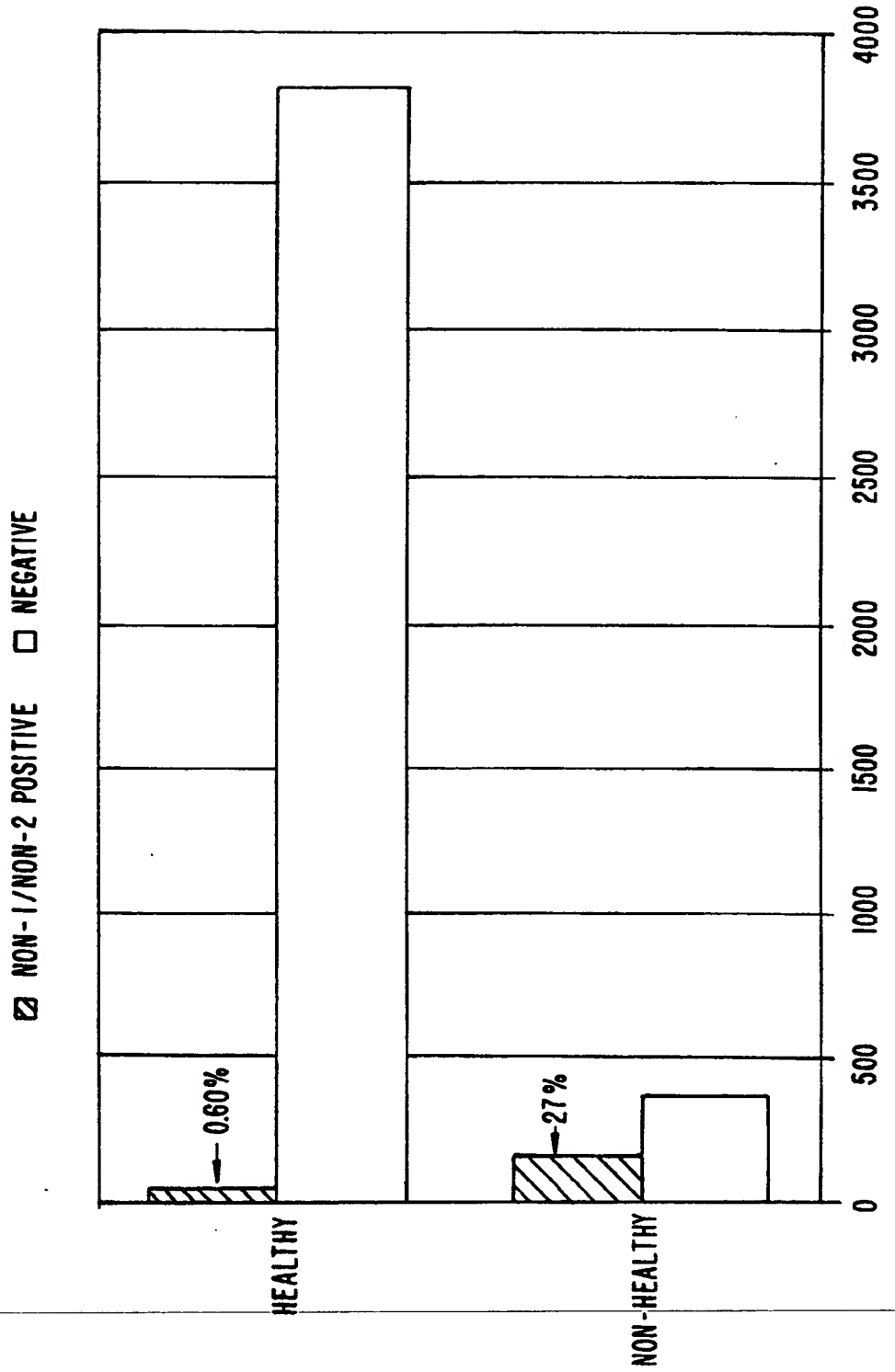
NON-1/NON-2 POSITIVE
NEGATIVE
NUMBER OF SUBJECTS
0
500
1000
1500
2000
2500
3000
3500
4000
0.60%
27%
HEALTHY
NON-HEALTHY

FIG. 1.

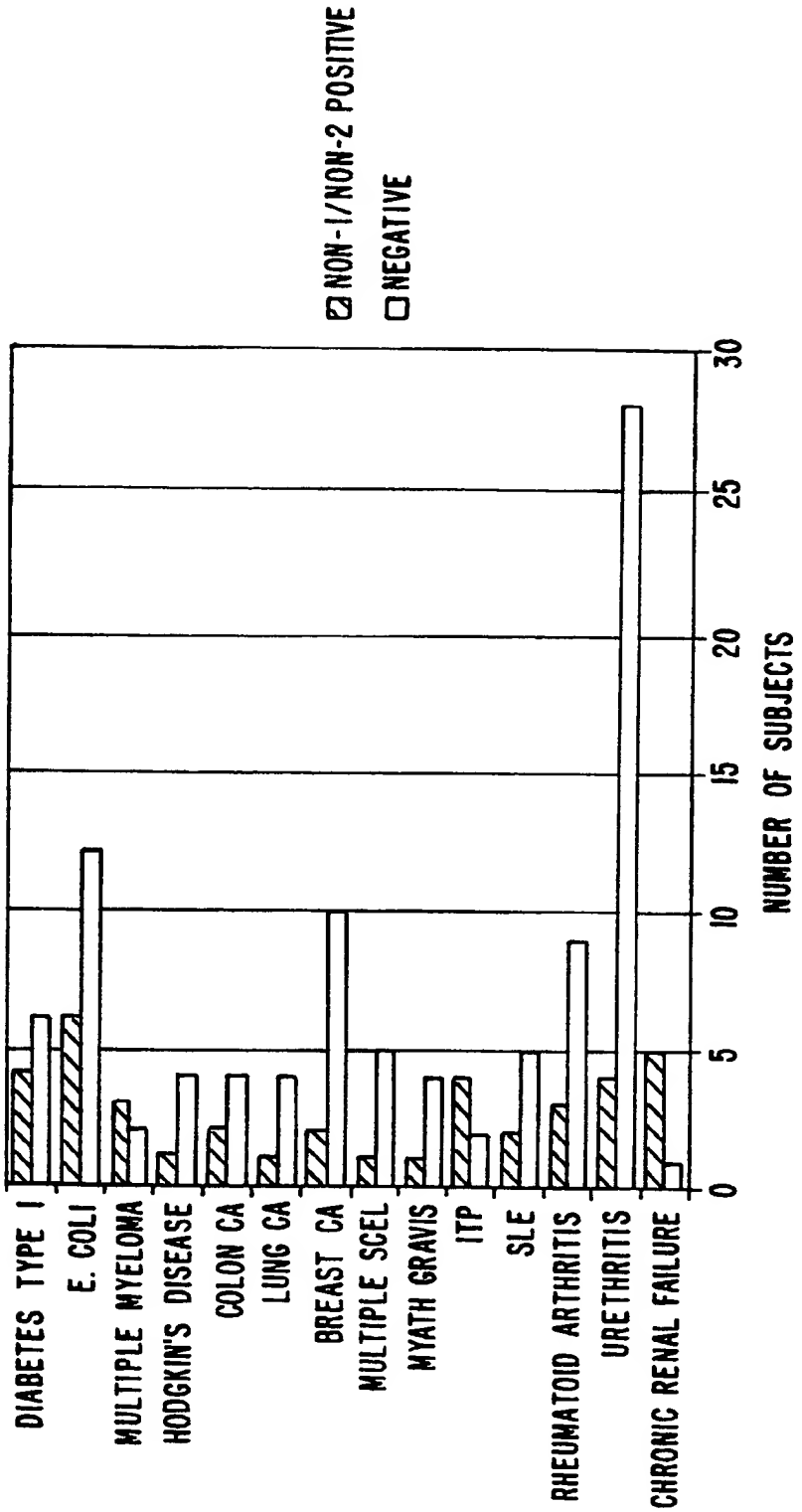
NON-1/NON-2 POSITIVE
NEGATIVE
NUMBER OF SUBJECTS
0
5
10
15
20
25
30
DIABETES TYPE I
E. COLI
MULTIPLE MYELOMA
HODGKIN'S DISEASE
COLON CA
LUNG CA
BREAST CA
MULTIPLE SCEL
MYATH GRAVIS
ITP
SLE
RHEUMATOID ARTHRITIS
URETHRITIS
CHRONIC RENAL FAILURE

FIG. 2.

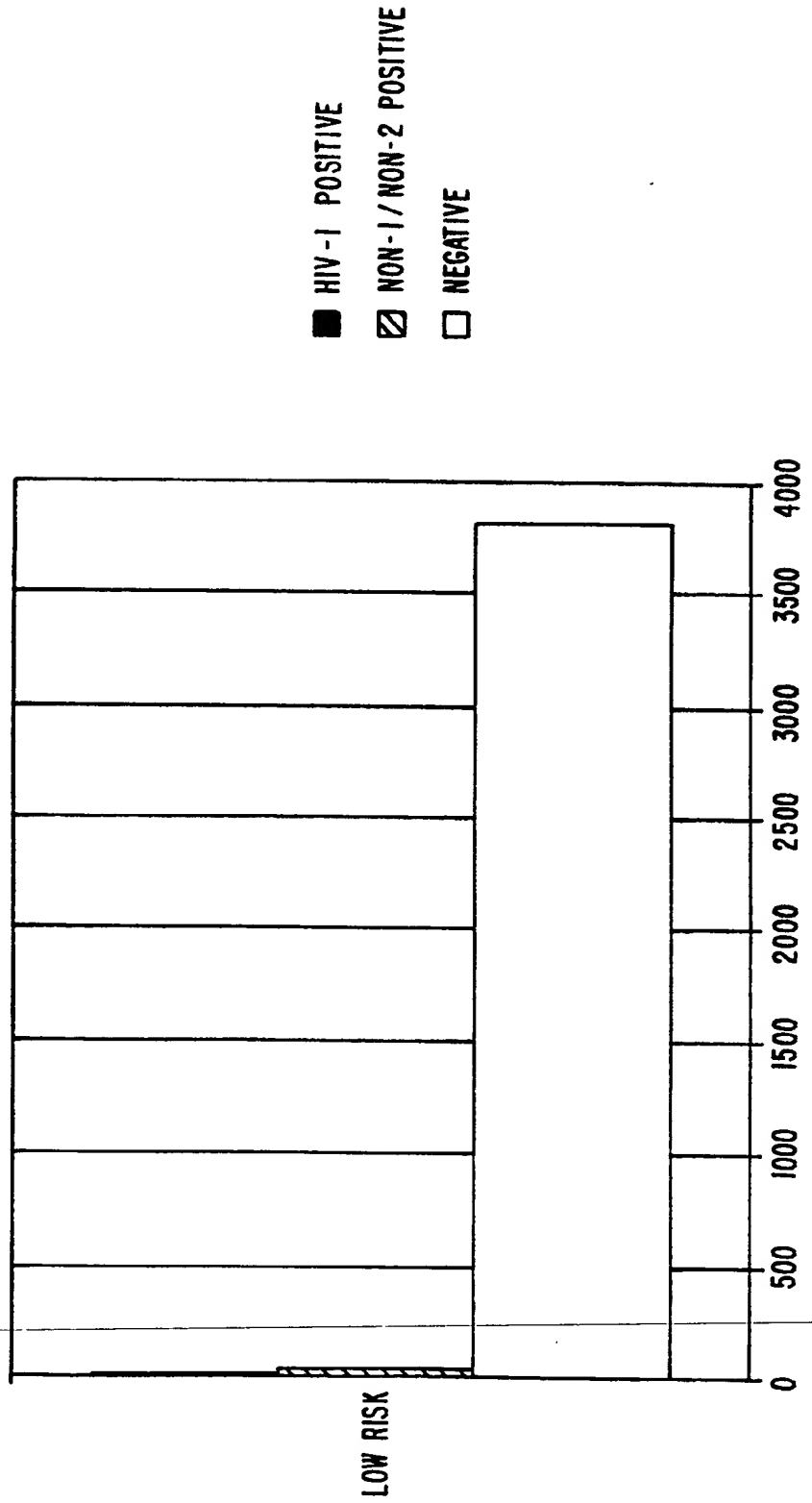
HIV-1 POSITIVE
NON-1/NON-2 POSITIVE
NEGATIVE
0
500
1000
1500
2000
2500
3000
3500
4000
NUMBER OF SUBJECTS
LOW RISK

FIG. 3.

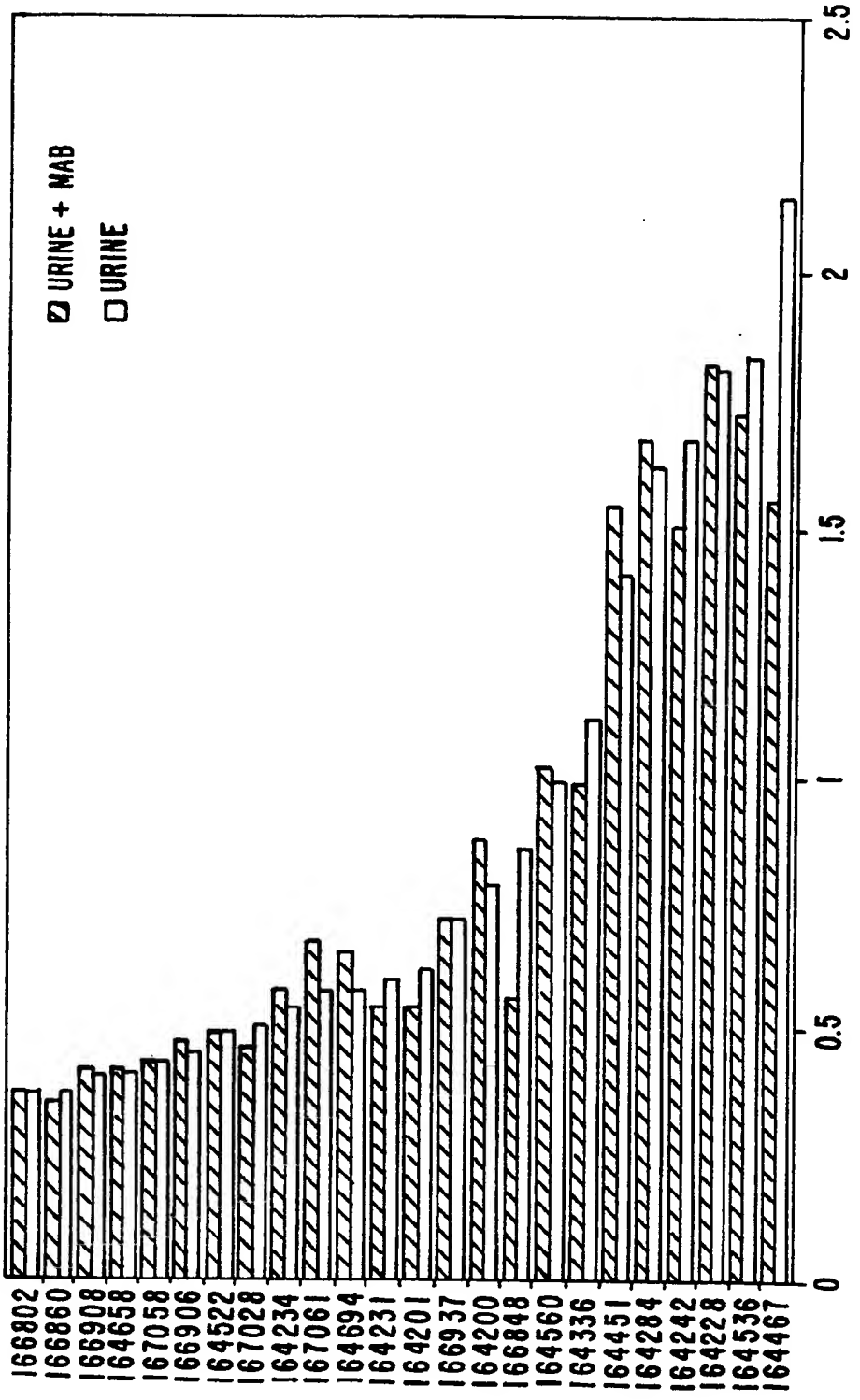
URINE + MAB
URINE
0
0.5
1
1.5
2
2.5
OD
166802
166860
166908
164658
167058
166906
164522
167028
164234
167061
164694
164231
164201
166937
164200
166848
164560
164336
164451
164284
164242
164228
164536
164467

FIG. 4.

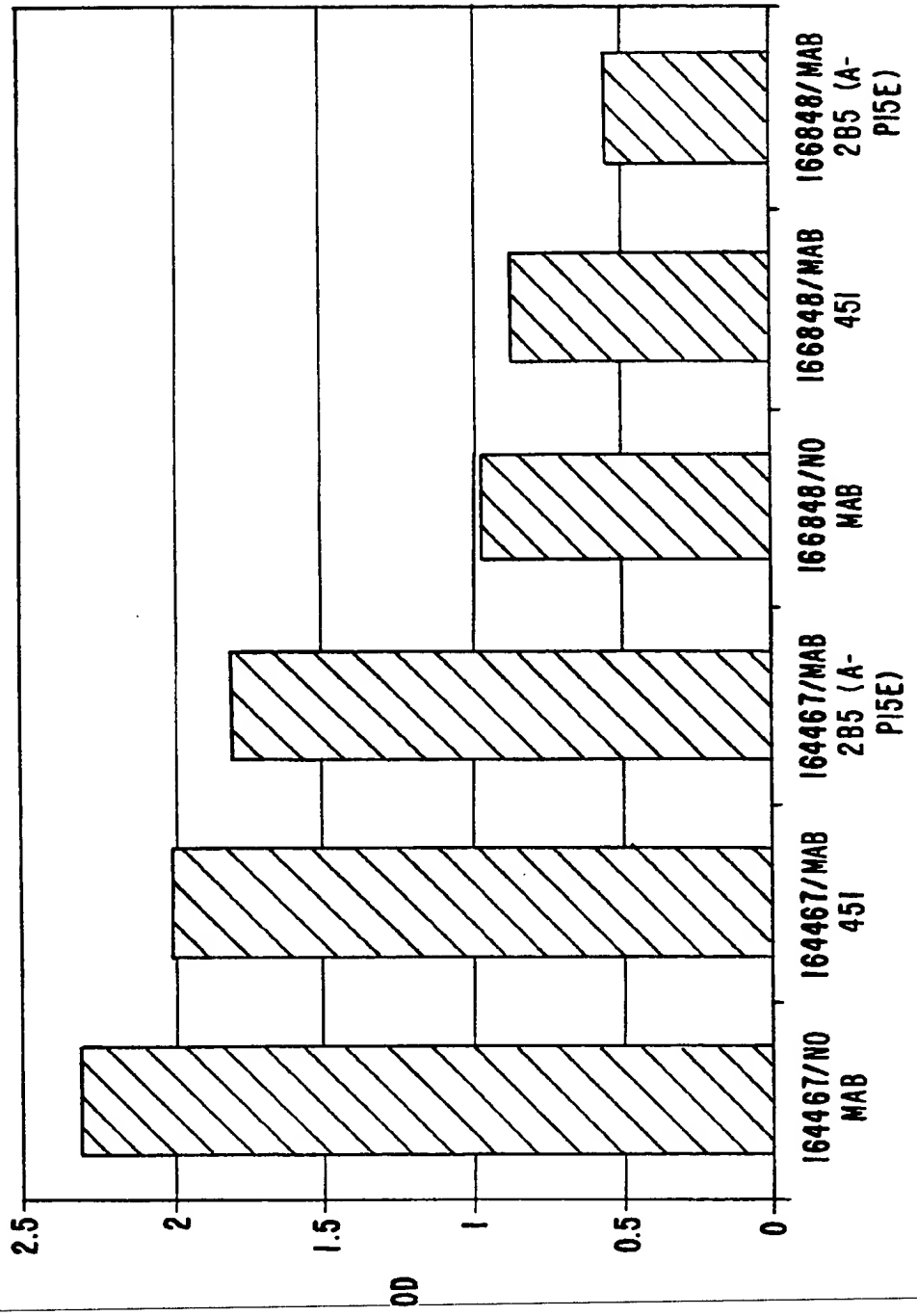
OD
2.5
2
1.5
1
0.5
0
164467/NO MAB
164467/MAB 451
164467/MAB 2B5 (A-P15E)
166848/NO MAB
166848/MAB 451
166848/MAB 2B5 (A-P15E)

FIG. 5.

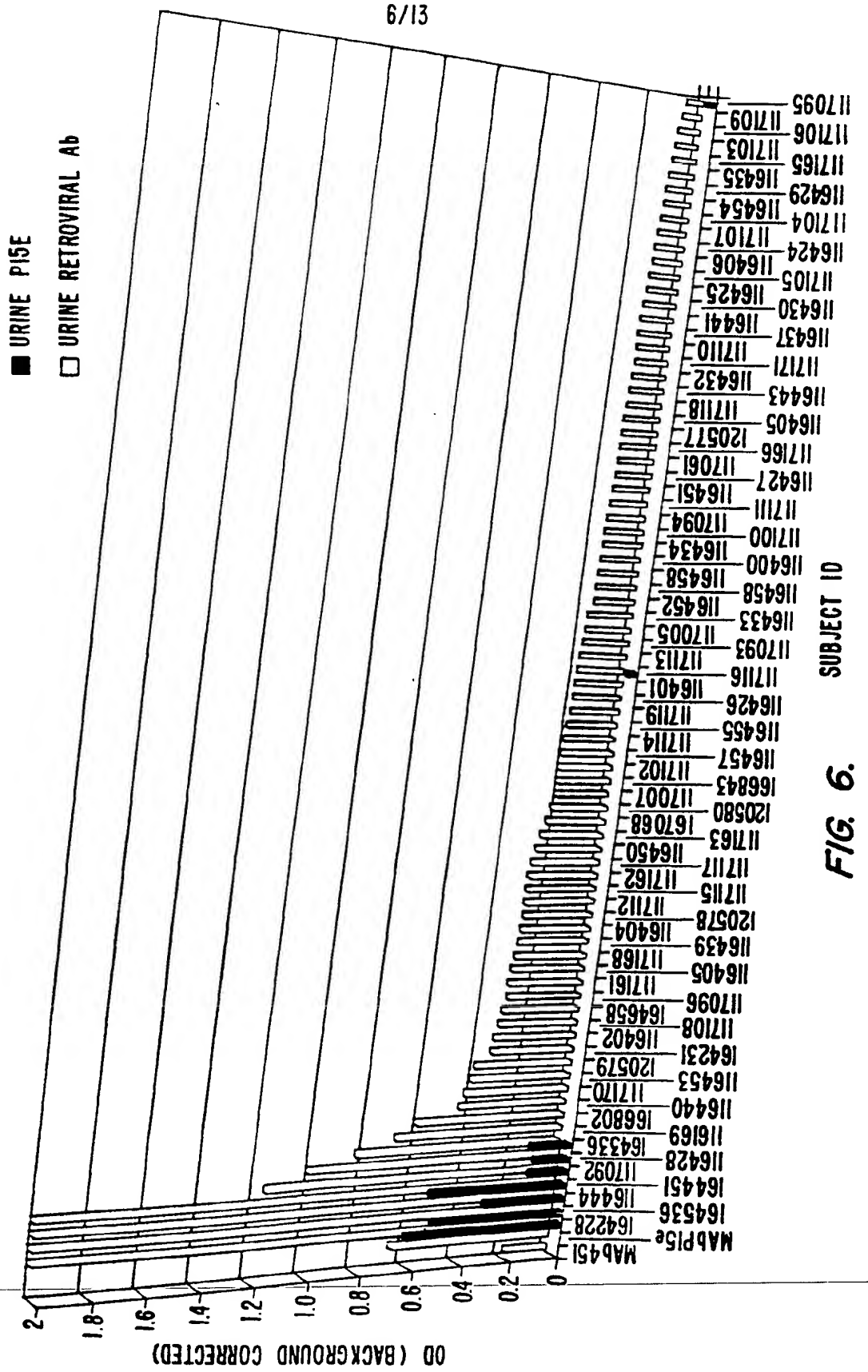
URINE P15E
URINE RETROVIRAL Ab
OD (BACKGROUND CORRECTED)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2
SUBJECT ID
MAbP15e
MAb451
164228
164536
164451
164444
117092
164428
116169
166802
116440
117170
116453
120579
164231
116402
117108
164658
117096
116405
117161
117168
116439
116404
120578
117112
117115
117162
117117
116450
117163
167068
120580
117007
166843
117102
116457
117114
116455
117119
116426
116401
117116
117113
117093
117005
116433
116452
116458
116458
116400
116434
117100
117094
117111
116451
116427
117061
116677
120577
116405
117118
116443
116432
117171
117110
116437
116441
116430
116425
117105
116406
116424
117107
117104
116454
116429
116435
117165
117103
117106
117109
117095

FIG. 6.

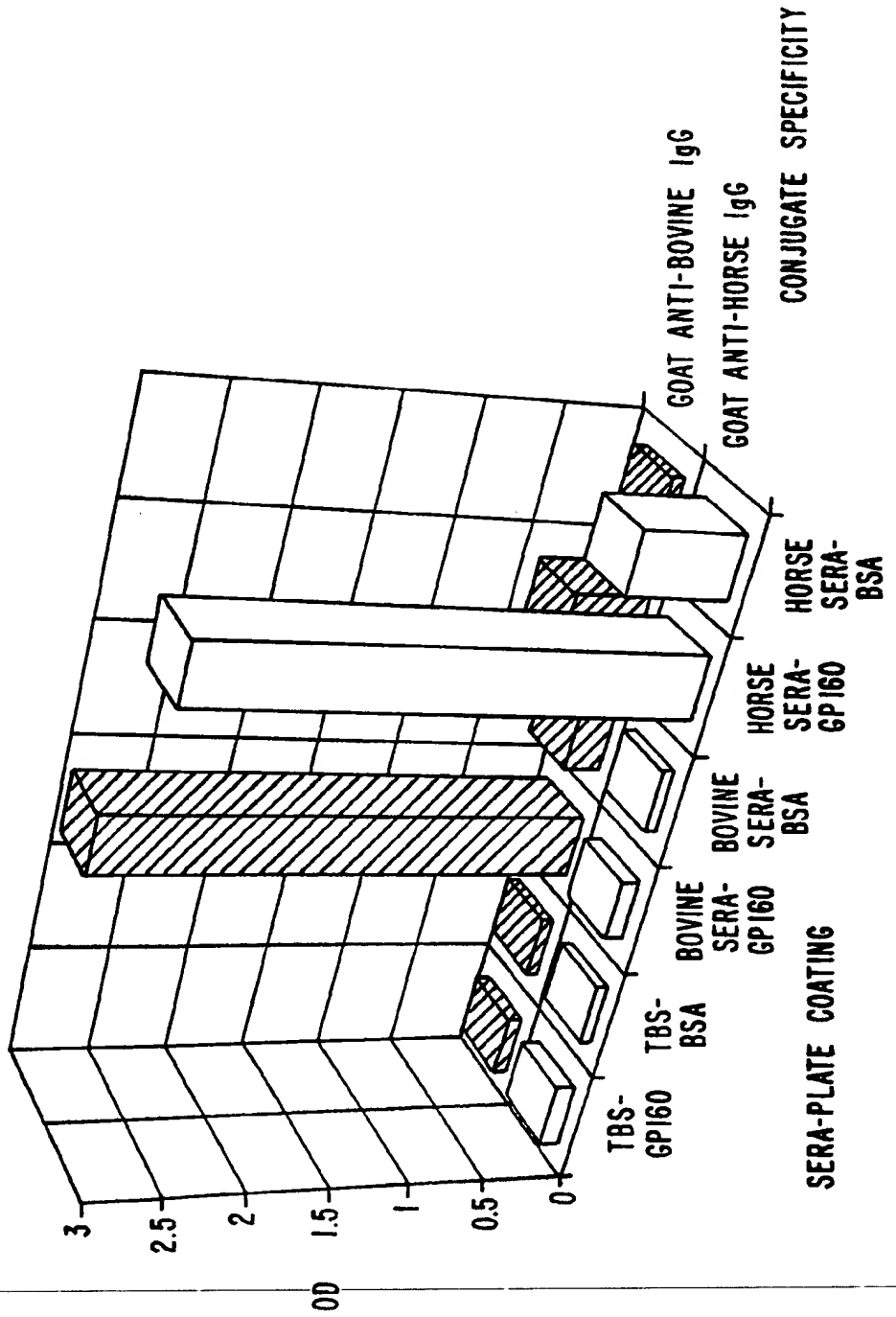
OD
3
2.5
2
1.5
1
0.5
0
SERA-PLATE COATING
TBS-GP160
TBS-BSA
BOVINE SERA-GP160
BOVINE SERA-BSA
HORSE SERA-GP160
HORSE SERA-BSA
CONJUGATE SPECIFICITY
GOAT ANTI-HORSE IgG
GOAT ANTI-BOVINE IgG

FIG. 7.

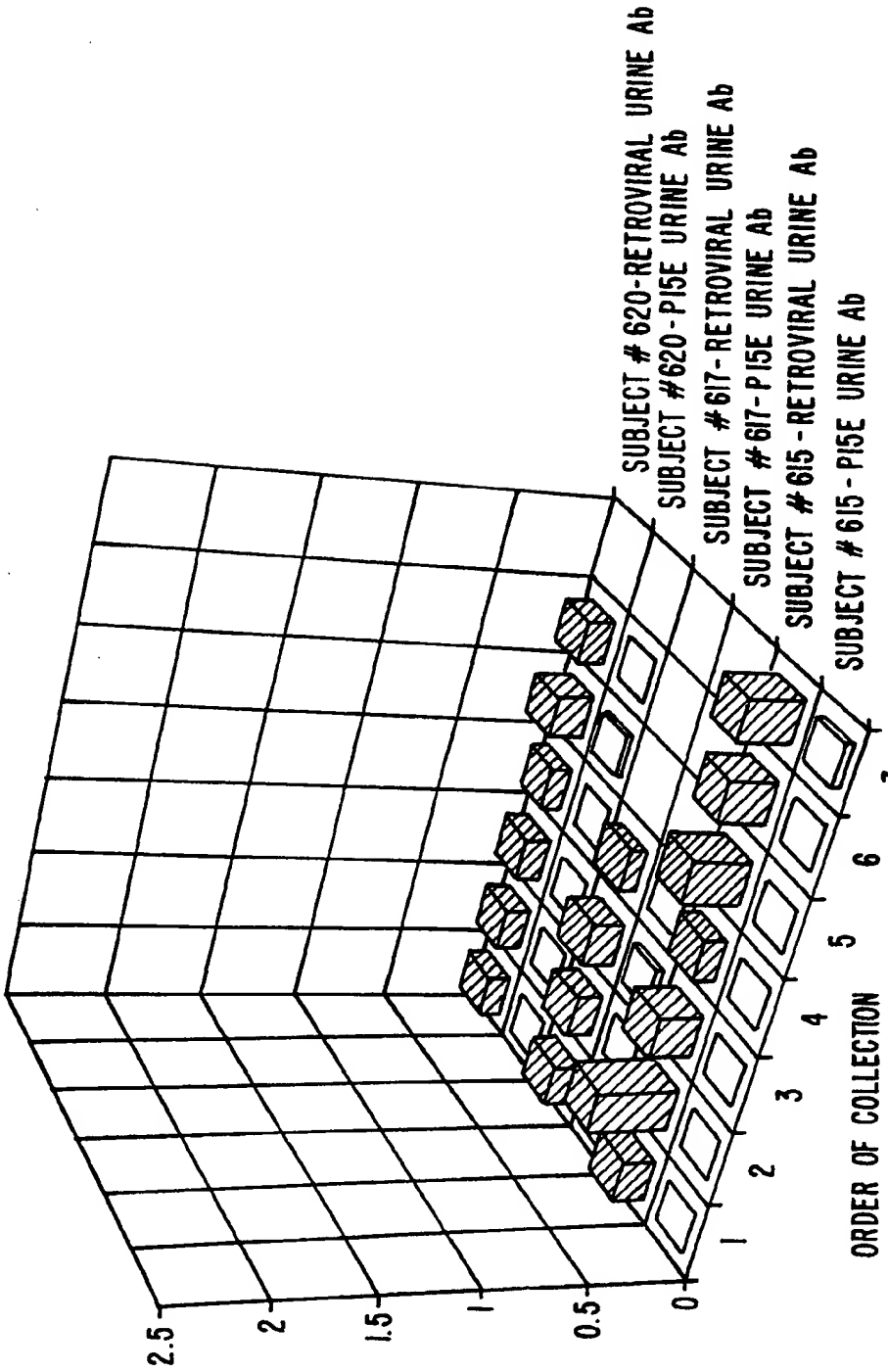
SUBJECT # 620-RETROVIRAL URINE Ab
SUBJECT # 620-P15E URINE Ab
SUBJECT # 617-RETROVIRAL URINE Ab
SUBJECT # 617-P15E URINE Ab
SUBJECT # 615-RETROVIRAL URINE Ab
SUBJECT # 615-P15E URINE Ab
ORDER OF COLLECTION
1
2
3
4
5
6
7
OD (BACKGROUND CORRECTED)
0
0.5
1
1.5
2
2.5

FIG. 8.

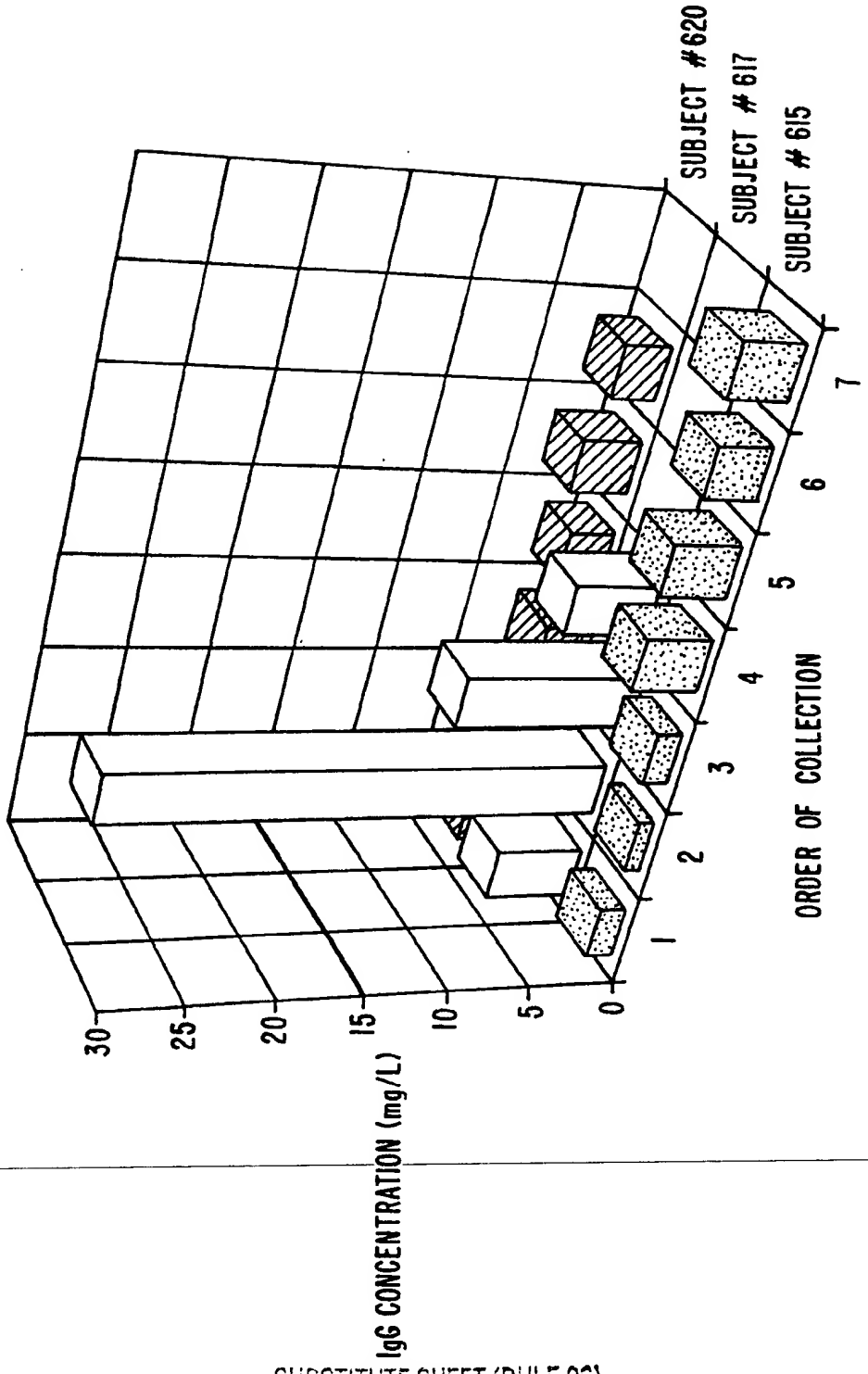
SUBJECT #620
SUBJECT #617
SUBJECT #615
ORDER OF COLLECTION
1
2
3
4
5
6
7
IgG CONCENTRATION (mg/L)
0
5
10
15
20
25
30

FIG. 9.

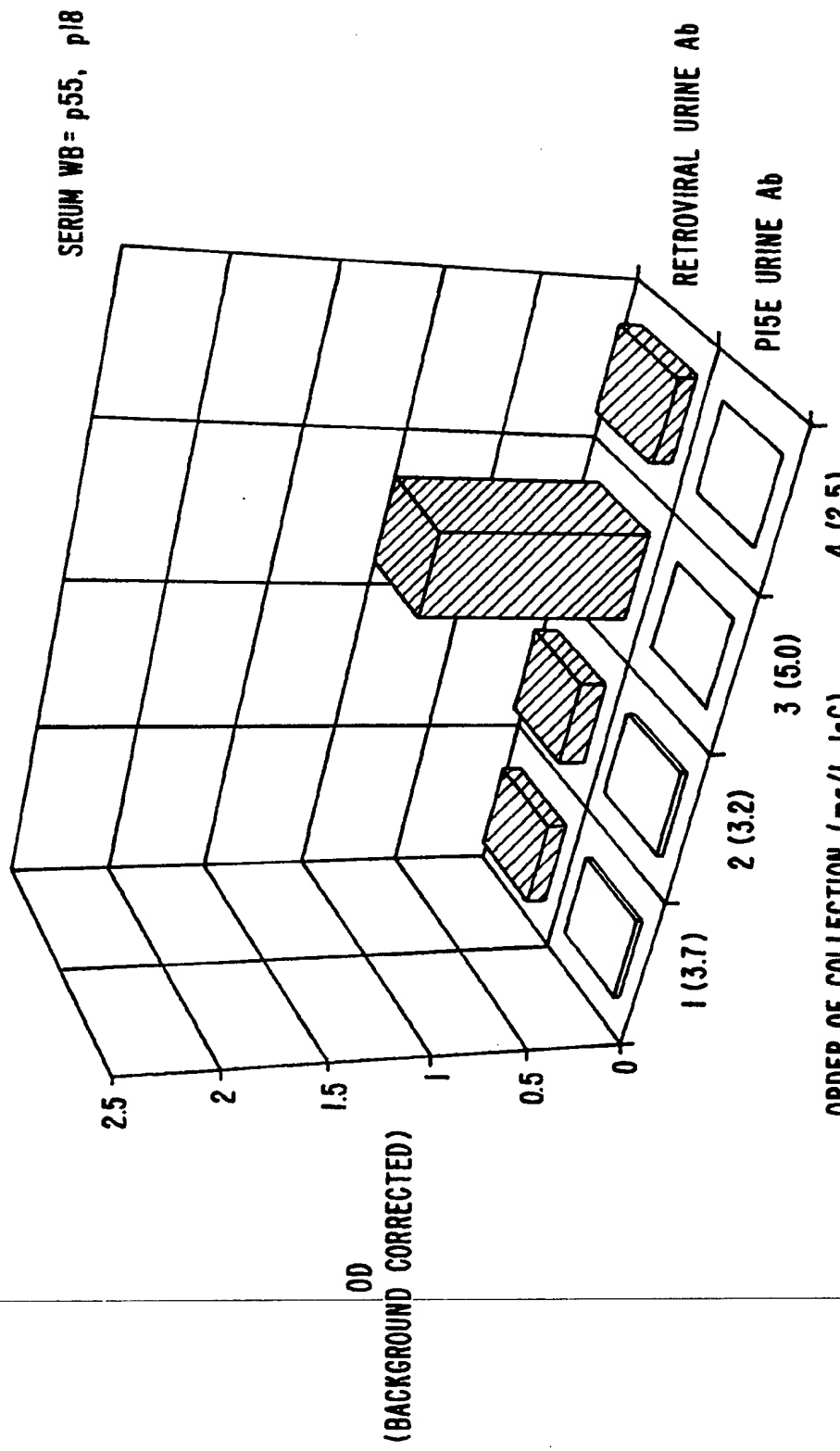
SERUM WB = p55, p18
RETROVIRAL URINE Ab
PI5E URINE Ab
OD
(BACKGROUND CORRECTED)
2.5
2
1.5
1
0.5
0
1 (3.7)
2 (3.2)
3 (5.0)
4 (2.5)
ORDER OF COLLECTION (mg/L IgG)

FIG. 10.

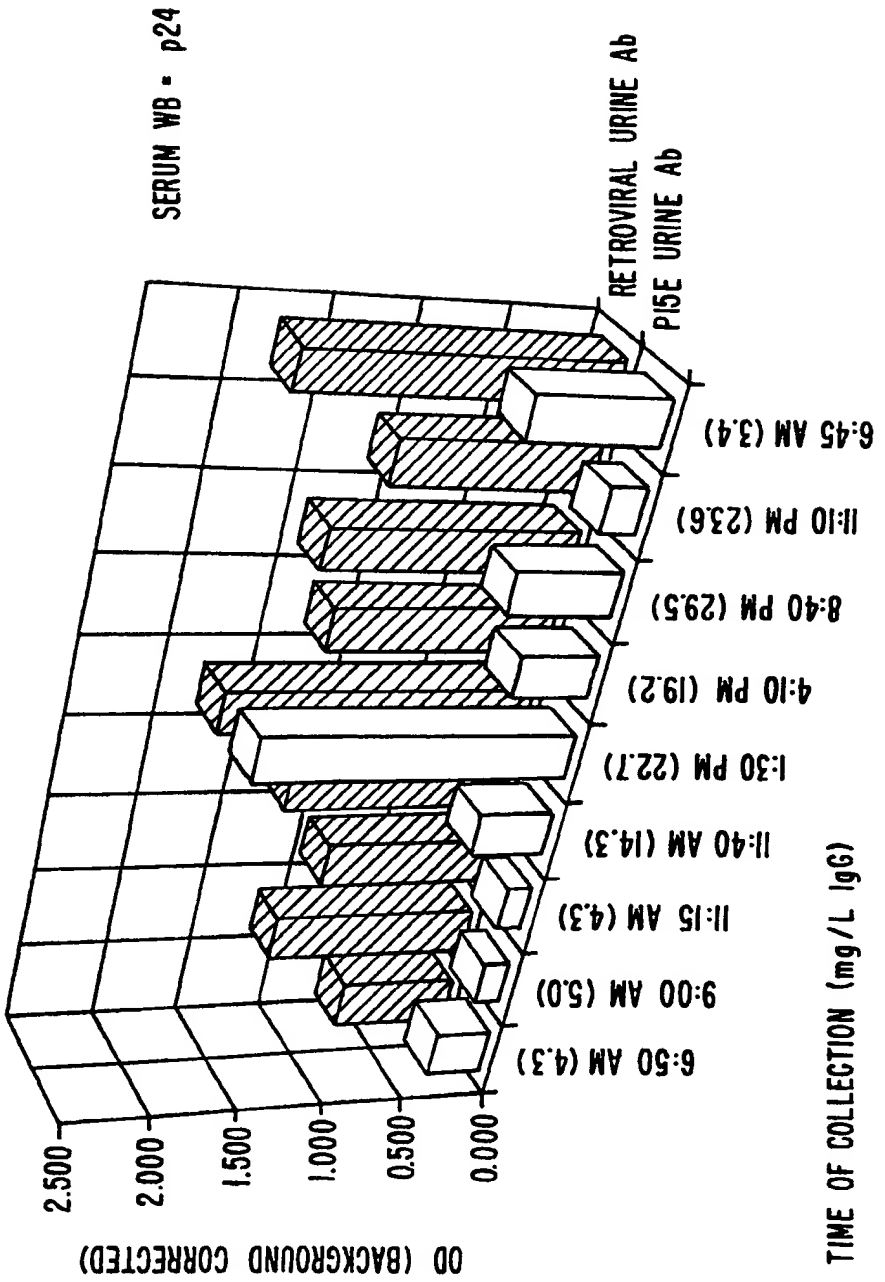
SERUM WB - p24
RETROVIRAL URINE Ab
P15E URINE Ab
6:45 AM (3.4)
11:10 PM (23.6)
8:40 PM (29.5)
4:10 PM (19.2)
1:30 PM (22.7)
11:40 AM (14.3)
11:15 AM (4.3)
9:00 AM (5.0)
6:50 AM (4.3)
TIME OF COLLECTION (mg/L IgG)
2.500
2.000
1.500
1.000
0.500
0.000
OD (BACKGROUND CORRECTED)

FIG. 11.

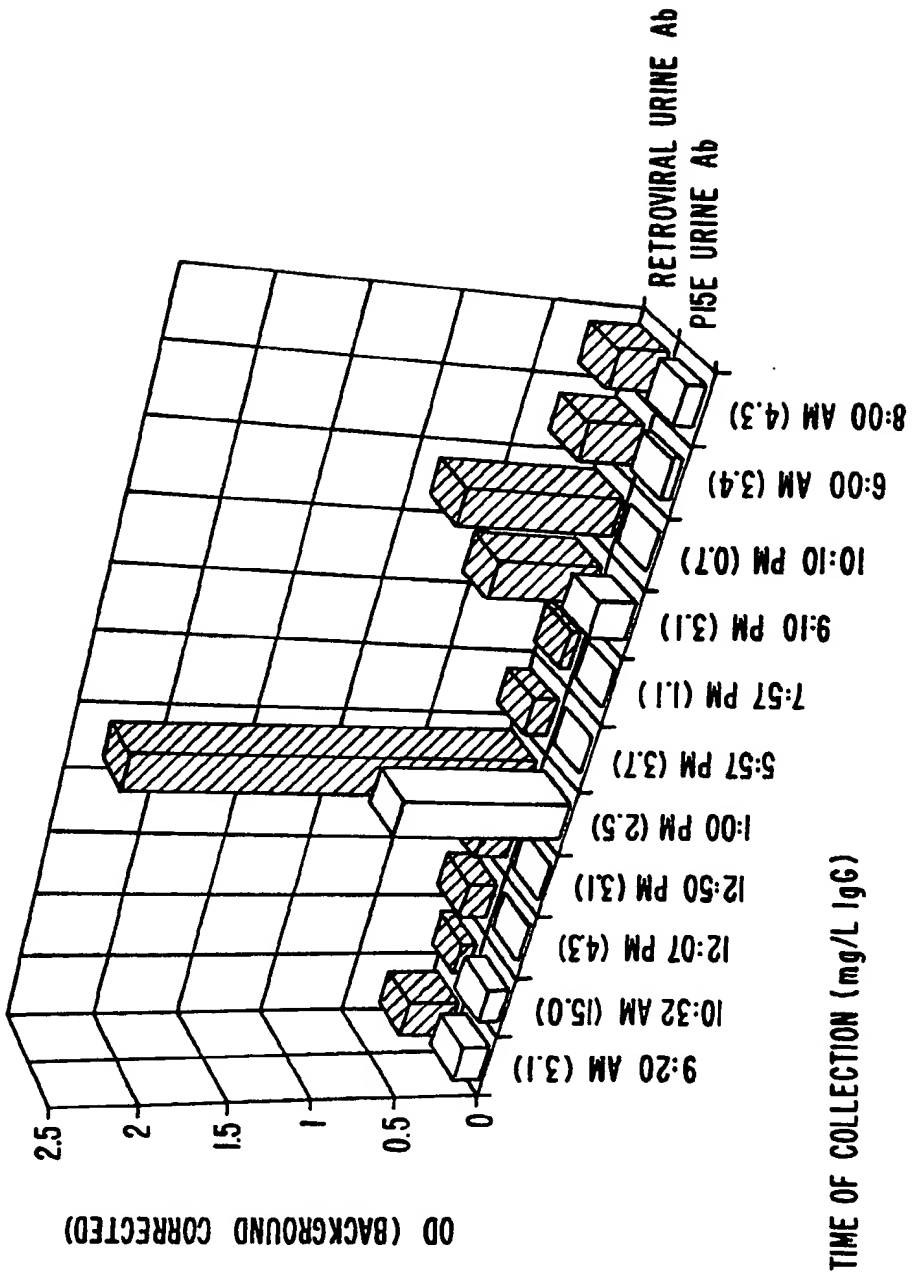
RETROVIRAL URINE Ab
PISE URINE Ab
8:00 AM (4.3)
6:00 AM (3.4)
10:10 PM (0.7)
9:10 PM (3.1)
7:57 PM (1.1)
5:57 PM (3.7)
1:00 PM (2.5)
12:50 PM (3.1)
12:07 PM (4.3)
10:32 AM (15.0)
9:20 AM (3.1)
TIME OF COLLECTION (mg/L IgG)
2.5
2
1.5
1
0.5
0
OD (BACKGROUND CORRECTED)

FIG. 12.

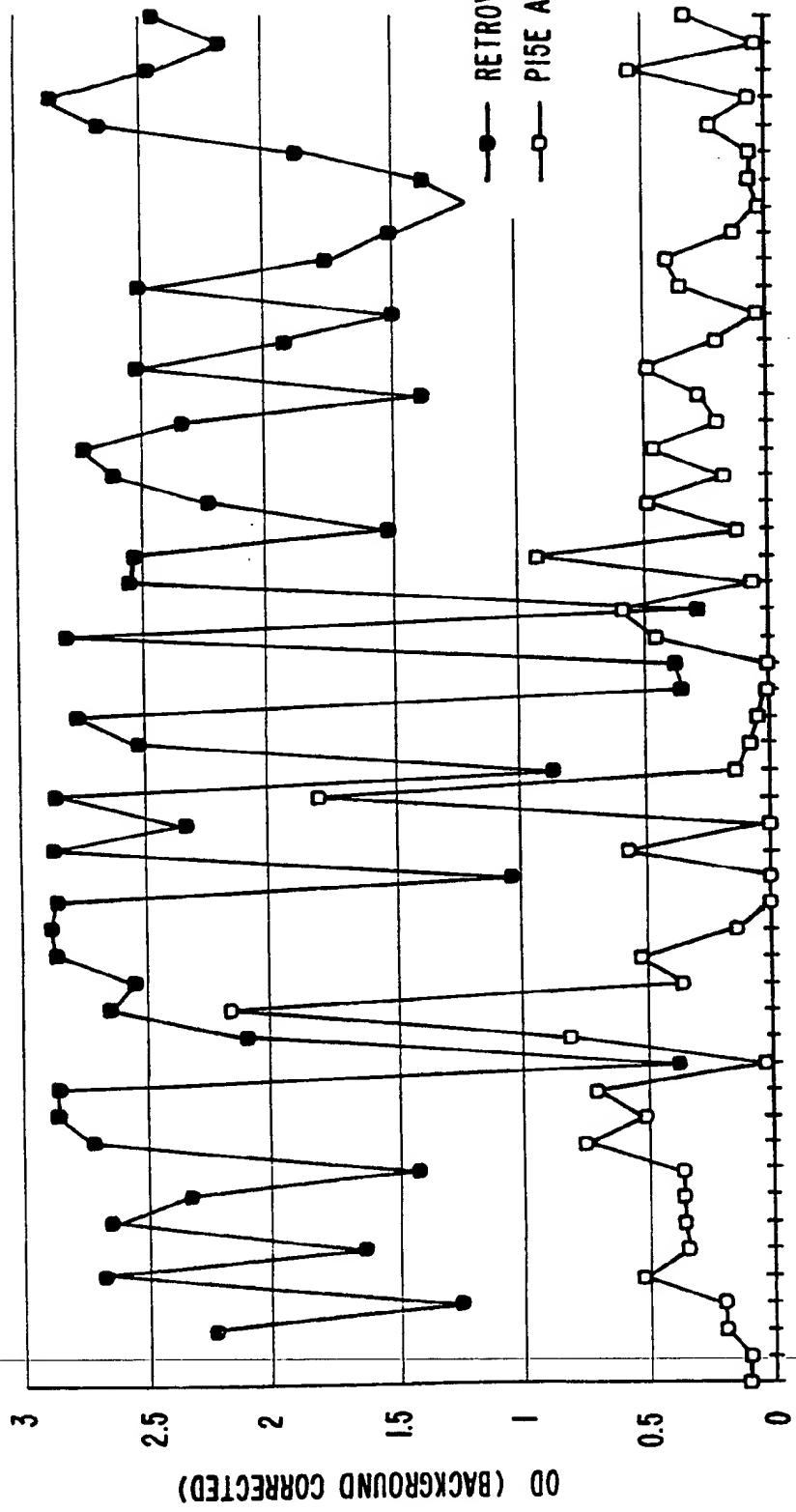
RETROVIRAL Ab
P15E Ab
OD (BACKGROUND CORRECTED)
3
2.5
2
1.5
1
0.5
0
DAY OF COLLECTION

FIG. 13.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/06549

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12Q 1/70
US CL :435/5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/5; 435/506, 811, 813

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P — Y	US, A, 5,344,774 (GARRY ET AL.), 6 September 1994, see entire document, especially col. 3, lines 7-11.	1, 10, 23 — 2-9, 11-22, 24-27
Y, P	US, A, 5,320,940 (TALAL ET AL.), 14 JUNE 1994, see entire document.	1-27
Y	US, A, 4,822,606 (SNYDERMAN ET AL.) 18 April 1989, see entire document.	1-27
Y	Science, Vol. 250, issued 23 November 1990, R.F. Garry et al., "Detection of a Human Intracisternal A-Type Retroviral Particle Antigenically Related to HIV," pages 1127-1129, see entire document.	1-27

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 AUGUST 1995	29 AUG 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DONNA C. WORTMAN, Ph.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/06549

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Annals of Allergy, Volume 69, issued September 1992, N. Talal et al., "Evidence for possible retroviral involvement in autoimmune diseases," pages 221-224, see entire document.	1-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/06549

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-27

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/06549

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-27, drawn to a method of detecting and kit.
Group II, claims 28-39, drawn to a treatment method.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The claims of Group II are drawn to a treatment method which is distinct from the detection method of Group I because the in vivo treatment method requires different process steps and has different goals and outcomes from the in vitro detection method of Group I. PCT Rule 13 does not provide for multiple methods of use of a single product.